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THE EFFECT ON THE COMPOSITION OF THE BLOOD OF MAINTAINING AN INCREASED BLOOD VOLUME BY THE INTRAVENOUS INJECTION OF A HYPERTONIC SOLUTION OF GUM ACACIA AND GLUCOSE IN NORMAL, ASPHYXIATED AND SHOCKED DOGS

H. L. WHITE AND JOSEPH ERLANGER

From the Physiological Department of Washington University, St. Louis

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The present work was undertaken with the idea of investigating the effect upon the composition of the blood of producing and then maintaining for some hours an expansion of the blood volume through the intravenous injection of a hypertonic crystalloid and colloid in combined solution, in the hope of throwing some light on the mechanism of the processes involved. The solution injected consisted of 18 per cent glucose and 25 per cent gum acacia and is the one that has been found useful in the treatment of traumatic shock (1). Studies of the blood changes produced were carried out under conditions which were varied in a way that might change the permeability of the vessel walls to the tissue fluids and their various constituents.

The literature contains many references to studies, more or less complete, of the blood changes following the intravenous injection of crystalloids, glucose, NaCl, Na_2SO_4 and urea being the substances most commonly used. Brasol (2) found that following intravenous injection of a hypertonic glucose solution the blood was greatly diluted in 2 minutes but that the blood volume had returned to normal within 2 hours. He found that there was no relation between the amount of glucose injected and the per cent of glucose found in the blood 2 minutes later, that the blood sugar was normal 2 hours after the injection, that part of the sugar which leaves the blood can be found in the tissues, but

part cannot be found and is perhaps changed to glycogen, lactic acid or "undergoes some other chemical metamorphosis," and that the absolute quantity of protein in the serum remains unchanged. He concludes that the effects produced are purely the results of the increased intravascular osmotic tension due to the injected glucose. Klickowicz (3) obtained similar results as regards blood volume with strong solutions of Na_2SO_4. Leathes (4) finds that the "increase in the volume of the blood caused by the injection of 5 grams of dextrose per kilo is enormous and quite out of proportion to that caused by the volume of the injection. This increase takes place with remarkable rapidity; the volume of the blood is nearly doubled by the time the injection is finished." Gasser and Erlanger (5) followed the blood volume after intravenous injection of 5 cc. of 18 per cent glucose solution per kilo body weight, the injection being made as rapidly as possible. They found that the maximum dilution was attained within 0.5 to 2 minutes and that the blood then began to concentrate, rapidly at first, and then more slowly, and became normal within 5 to 45 minutes. Starling (6) finds hydremic plethora following glucose injection and that the distended vessels begin at once to unload the excess of water. Paton (7) finds that when 4 grams of carbohydrate in 10 cc. of water per kilo body weight are injected intravenously only a small per cent of the amount injected can be recovered immediately from the blood, the time from the beginning of the injection to the end of collecting the sample being 90 to 100 seconds. Hamburger (8) finds that in the horse an intravenous injection of 7 liters of 5 per cent Na_2SO_4 causes a marked dilution of blood serum in respect to its chloride and protein content, a return to normal being accomplished very quickly, within 2 hours. Practically the same effect on protein content of serum follows the injection of 5 liters of 0.5 per cent Na_2SO_4 solution, while the chlorides are diluted as with a hypertonic solution but do not return to normal. The blood volume was not followed in these cases, so no conclusions can be drawn as to whether the return to normal was due to a passage of water out of the blood stream or an entrance of solids into the blood stream. Fisher and Wishart (9) found that blood dilutes as a result of alimentary hyperglycemia. Magnus (10) obtains varying results in his studies of changes in blood composition following the intravenous injection of NaCl solutions, the results depending upon the osmotic tension of the injected solution, although consistent results were not obtained with hypertonic solutions.

The common feature of the experiments discussed above is that the substances injected were crystalloids and, with the exception of those of Fisher and Wishart, the injection was rapid; and the increase in blood volume, with hypertonic solutions, always is very rapid in appearance and of short duration. Woodyatt, Sansum and Wilder (11) injected glucose intravenously over long periods of time at a uniform rate and determined the tolerance rate as 0.8 to 0.9 gram per kilo per hour. Erlanger and Woodyatt (12) injected glucose intravenously at uniform rates varying between 0.57 and 4 grams per kilo per hour for from 20 to 60 minutes into anesthetized animals in shock. The pulse amplitude was uniformly markedly increased, indicating a condition of plethora. A subtolerant dose was as effective as injections made at more rapid rates. In a normal animal an injection lasting 30 minutes at the rate of 1.78 gram per kilo per hour raised arterial pressure only about 5 mm. Hg. but the pulse amplitude increased quite appreciably. The fact that a considerable increase in blood volume is accompanied by only a slight rise in arterial pressure is presumably due to diminution in viscosity of the blood and to vasomotor accommodation. While the blood volume was not followed in these cases it presumably soon fell after cessation of injection to its pre-injection state.

With regard to the entrance of protein into the circulation, Morawitz (13) found restoration of proteins most active in the first few hours after severe hemorrhage. Whipple and co-workers (14) find that after severe hemorrhage the regeneration of plasma proteins is much more rapid on a liberal high protein diet than on a liberal bread and milk diet, and least rapid on fasting. They infer from this that there is an actual new formation of protein and make no mention of the possibility of some of the protein being drawn in from the tissue fluids. They also find that "after the initial depletion of serum proteins the body can almost always regenerate 1 per cent of the total protein during the first 24 hours following the plasmapharesis. This figure is remarkably constant and does not seem to be influenced by diet or fasting . . . . . or the amount of shock. We may perhaps look upon this as the maximum effort of the body to replace these essential serum proteins, an effort which surely depends upon the body protein as it is present in fasting experiments. It will be the same whether there is a marked breakdown of host protein, as evidenced by great increase in urinary nitrogen, or whether this tissue autolysis is minimal." That they do not regard this maximum effort as accomplished by the passage of protein directly into the blood from the tissues but rather as a true

rebuilding is shown by their further statement that it "probably represents the absolute maximum production under the greatest stimulus." They present further evidence that the restoration of plasma proteins after hemorrhage is due to a new formation of proteins and that the liver is the chief seat of this process in that restoration is greatly hindered by phosphorus and chloroform poisoning and by an Eck fistula.

A rather careful review of the literature has failed to disclose any references to studies of the body fluids made during a period of prolonged increase of blood volume due to the injection of hypertonic solutions. It is evident that only two means are at our disposal for maintaining an increased blood volume for a comparatively long period, several hours for instance, with fluids other than blood or blood plasma. One is by a continuous injection of a hypertonic or isotonic solution of crystalloids at a rate more rapid than the rate of the blood's fluid loss. In this case the large amount of fluid injected would so dilute the blood that no conclusions could be drawn as to the interchange of water and solutes between tissue spaces and blood stream. The other method is to inject a small volume of a strongly hypertonic solution which will not only draw fluid into the blood stream but hold it there for some time. To accomplish this result a solution containing both crystalloid and colloid is necessary (15). So far as we know the present work represents the first effort made to study changes in the composition of the blood induced in this way.

PROCEDURE

Dogs were used in all experiments. The animals were not prepared by controlling their intake of food or water previous to the experiment. Most of them were fed and watered on the morning of the experiment. In the first seven cases the animal was given 1 grain of morphine an hour before starting the operation; in the case of dog 8, 2 grains were given by mistake. In the remainder of the cases the morphine was omitted because of its disturbing effect on the blood and urinary sugar. In the first four cases strict surgical asepsis was observed throughout the experiment; in the remainder these precautions were relaxed, although the fluid injected and the injecting apparatus were sterile. No differences in results due to these technical differences were observed. Three sets of experiments were performed; the first, a series of nine experiments, on normal dogs; the second, a series of two, on asphyxiated dogs; the third, a series of three, on dogs in shock. The procedure and data of each series are presented separately.

The *procedure in the first series* was to anesthetize the dog with ether, immediately draw a "standard" sample of blood from the femoral artery, then to start the injection into the femoral vein. The dose in all cases was 5 cc. per kilo per hour of an 18 per cent glucose and 25 per cent gum acacia solution, the injection lasting 1 hour and proceeding at a uniform rate. Immediately at the termination of the injection another sample of blood was taken and the dog put back into its cage. Subsequent samples were taken at approximately 2-hour intervals until five samples were obtained. Little or no anesthetic was required while drawing the last three samples. On each sample determinations were made of hemoglobin (16), plasma chlorides (17), plasma total nitrogen and plasma non-protein nitrogen (18), and plasma sugar (18). The nitrogen and chloride determinations were made in duplicate. Direct Nesslerization was at first attempted for both the total and the non-protein nitrogen determinations, appropriate dilutions being made, but it was found that the presence of the large amount of carbohydrate in the samples rendered digestion with the prescribed digestion mixture so difficult that indirect Nesslerization had to be resorted to. In the latter cases of the series the total nitrogen determinations were made by the macro-Kjeldahl method since it was felt that the micro-Kjeldahl with indirect Nesslerization was not sufficienty trustworthy. Figures given in the tables under the heading "total N" are protein nitrogen figures and are obtained by subtracting the non-protein nitrogen figure from the total nitrogen figure as determined by the Kjeldahl. Several dilutions of the filtrate for plasma sugar determinations were made, the dilution being accepted whose reading was closest to 20, the standard being set at 20 on the Dubosq or Bock-Benedict colorimeter. Freezing point determinations were attempted in the first experiment, working with 1.5 cc. of hirudinized plasma in a special tube in the Beckmann apparatus, but it was found that consistent results could not be obtained with this amount of plasma and these determinations were discontinued as it seemed inadvisable to take more blood. In lieu of direct determinations the crystalloid osmotic pressure of the plasma has been roughly estimated by adding the osmotic pressures exerted by the NaCl and glucose present. The calculations were made from published tables of direct osmotic pressure determinations and of freezing point determinations. By using various schemes for utilizing every available drop of plasma it was possible to make all determinations with 13 cc. of blood drawn at each sample. Allowance for blood drawn as samples has been made in the calculations. Dogs 8 and 9 were bled 15 per cent

of their blood volume at the time of taking the first sample. In the last few cases the urine was also followed, the bladder being emptied as completely as possible with a small coudé catheter at the time of drawing the blood samples. The results of the first series are given in table 1.

The *procedure in the second series* was to anesthetize the dog with ether (morphine being omitted in these and subsequent experiments), insert a tracheal cannula, draw a standard sample of blood from the femoral artery and empty the bladder. The animal was then made to rebreathe air in a large spirometer until the CO_2 tension of the air in it mounted to about 30 mm. Hg. as determined with Marriott's (19) tubes on samples drawn from the spirometer. The injection was then started, the CO_2 tension being kept around 30 to 35 mm. Hg., fresh air being admitted at intervals when the dog became markedly dyspneic. Dyspnea was moderate to marked throughout the injection. This procedure was introduced in an effort to discover whether asphyxia altered the permeability of the vessel walls. At the conclusion of the injection the second sample of blood and urine was drawn, rebreathing discontinued, the animal put back into its cage and subsequent samples taken as in the first series. The results of the second series are given in table 2.

In the case of dog 10 the injection rate was not accurately timed, at first. Twenty-five cubic centimeters were run in during the first 20 minutes. The injection was then stopped for 10 minutes, the remainder of the injection then proceeding at the proper rate. When the last sample was drawn from dog 10 he struggled quite vigorously and it was necessary to anesthetize him deeply, which is not usually necessary for the last three samples. This anesthesia probably accounts for the high blood sugar value in the last sample of dog 10.

The *procedure in the third series* was to anesthetize the animal, insert a tracheal cannula and connect a manometer with a femoral artery, draw a standard sample and then put the animal into a condition of shock. The condition induced was the so-called mechanical shock of Janeway and Jackson (20), which they produced by temporary partial occlusion of the vena cava regulated so as to keep the blood pressure at 30 to 40 mm. Hg. for 2 hours. The method of obstructing the cava used in the present work was one described by Erlanger and Gasser (21), a clamp being used by which graded compression can be exerted upon the cava between the liver and the diaphragm through a small abdominal incision. The mean arterial pressure was kept at about 40 mm. Hg. for 120 to 135 minutes. At the end of this time the clamp was removed, the second sample drawn, the injection given, a third sample

then taken, and the dog kept as long as he would live, samples being drawn at about 2- or 2½-hour intervals. Very little anesthetic was required to keep the dog quiet after shock had been produced. This procedure was employed in an endeavor to ascertain whether the permeability of the vessel walls is altered in shock.

The results of the third series are given in table 3 and in the brief case histories.

DISCUSSION OF RESULTS

Series I. Normal Dogs

1. Blood volume. It is evident that in all cases there is a marked immediate increase in blood volume, amounting to 11 to 16 per cent, which usually slowly returns toward normal. Some slight increase in volume, about 2 per cent, usually persists even after 6 or 7 hours. The combined action of the glucose and acacia in effecting changes in blood volume is interesting. The early high intravascular osmotic tension which causes the rapid increase in volume is due principally to the glucose. The glucose, however, rapidly passes out into the tissue spaces (see plasma sugar figures) but through the influence of the gum acacia the water very slowly leaves the vessels. The persistent increase of blood volume, 2 or more per cent, is less than the amount of water which must be added to the amount of acacia presumably remaining in the blood (22) in order to make it isosmotic to the plasma proteins. It will be noticed, however, that in every case but one, dog 3, where the figures are the same, the total amount of plasma proteins at the close of the experiment is less than that at the start. Part of the injected acacia, then, presumably is taking the place of the removed plasma protein in holding water, and only the remainder can be employed in holding an excess of water above normal blood volume. Quantitative determinations of acacia in the blood would be necessary in order to ascertain just how much acacia is available for maintaining an increased blood volume. Any fraction of the persisting increase in intravascular osmotic tension which might be due to the glucose could not play any part in maintaining the increase in blood volume, since glucose now exists in the tissue spaces in equal concentration, having passed out from the blood stream, and is exerting an equal attraction there. In fact, the blood sugar figures show that the excess of glucose has been entirely removed from the blood stream, and presumably from the tissue fluids, inside of 2 hours. The fate of the glucose will be discussed later.

TABLE 1

Data of series 1, normal dogs

DOG	SAMPLE	TIME	BLOOD VOLUME	PLASMA CHLOR.	SUGAR	N. P. N.	PROTEIN N				URINE		
							Remaining	Withdrawn	Total	Per 100 cc. plasma	Amount	Sugar	Sugar
		hours	*per cent*	*grams per 100 cc.*	*grams per 100 cc.*	*mgms. per 100 cc.*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*	*grams*
1	1		100	0.631									
	2	0	118	0.607									
	3	1 5′	112	0.619									
	4	3 15′	105	0.628									
	5	5 25′	106	0.629									
2	1		100	0.612	0.196	32							
	2	0	111	0.581	0.341	31							
	3	2	108	0.602	0.200	31							
	4	4	105	0.608	0.125	32							
	5	6 30′	103	0.610	0.127	31							
3	1		100	0.605	0.192	26	5.05		5.05	0.805			
	2	0	116	0.562	0.247	27	5.41	0.06	5.47	0.685			
	3	2	115	0.582	0.219	26	5.35	0.12	5.46	0.685			
	4	4 10′	113	0.589	0.127	25	5.21	0.18	5.39	0.685			
	5	6 15′	112	0.600	0.133	26	5.06	0.24	5.30	0.675			
4	1		100	0.595	0.282	32	4.53		4.53	0.837			
	2	0	114	0.537	0.564	33	4.11	0.06	4.17	0.622			
	3	2 20′	109	0.558	0.182	32	4.21	0.12	4.33	0.680			
	4	4 30′	105	0.572	0.182	33	4.31	0.18	4.49	0.742			
	5	7 25′	102	0.586	0.170	33	4.40	0.24	4.64	0.794			
5	1		100	0.579	0.232	25	3.94		3.94	0.720			
	2	0	116	0.533	0.381	25	no det.	0.06			6	4.8	0.29
	3	2 5′	113	0.554	0.178	26	3.79	0.12	3.91	0.572	8	6.0	0.48
	4	4 15′	109	0.570	0.167	25	3.89	0.18	4.07	0.623	35	3.2	1.12
	5	6 40′	102	0.574	0.172	26	3.50	0.24	3.74‡	0.624			
Total											49		1.89

6	1		100	0.635	0.222	33	6.46		6.46	0.852			
	2	0	113	0.601	0.500	32	6.08	0.07	6.15	0.660			
	3	2	112	0.618	0.217	33	6.09	0.14	6.25	0.674			
	4	4 25′	106	0.632	0.215	33	6.10	0.21	6.31	0.727			
	5	7 40′	101	0.635	0.204	32	5.96	0.28	6.24	0.772	245		5.6
Total											245	2.3	5.6
7	1		100	0.582	0.308	31	4.35		4.35	0.833			
	2	0	111	0.560	0.541	30	4.54	0.07	4.61	0.744			
	3	2 10′	106	0.568	0.342	31	4.42	0.14	4.56	0.760			
	4	4 20′	102	0.574	0.250	31	4.16	0.21	4.37	0.774			
	5	7	98	0.579	0.185	30	4.06	0.28	4.34	0.800	20	2.3	0.46
Total											20		0.46
8*	1		100	0.642	0.400	28	10.01		10.01	1.14	21	1.7	0.35
	2	0	99	0.610	0.606	27	8.72	1.555	10.27	1.03	108	7.1	7.7
	3	2	95	0.630	0.286	28	8.55	1.64	10.19	1.062	80	6.4	5.1
	4	4 10′	89	0.636	0.234	27	7.88	1.73	9.61	1.091	28	4.3	0.55
	5	6 20′	86	0.640	0.220	27	7.54	1.82	9.36	1.115	55	1.0	0.55
Total after injection											271		14.55
9†	1		100		0.133						200		0
	2	0	106		0.292						10		f. t.
	3	2	97		0.163						14		0.17
	4	4	94		0.133						20		tr.
	5	6 10′	93		0.135						42		0
Total after injection											86		0.17+

* Dog 8 bled 220 cc. at 1st sample.
† Dog 9 bled 160 cc. at 1st sample.
‡ Evidently some mistake in last determination.

The rather wide variations in the rate of the falling off from the initial increase in blood volume possibly are to be attributed to variations in vasomotor accommodation and in the preexisting water content of the tissue spaces. Scott (23) finds the hemoglobin content increased immediately by a rise in blood pressure produced by various procedures, and lowered by a fall in pressure. He cites evidence indicating that the hemoglobin method gives an accurate picture of relative blood volume; and, as he says, "these results can only be explained by increased pressure forcing fluid out of the blood to the tissue spaces and the passage of fluid back from the tissue spaces to the blood when the pressure is lowered." He did not determine the nature of the interchanged fluid.

At the time of taking the first sample dog 8 was bled approximately 15 per cent of his calculated blood volume, 15 per cent having been about the average initial increase in blood volume following the injection. This was done in order to ascertain whether the interchange of liquids and solids between the blood and the tissues is modified when the plethora that is induced by the injection of a hypertonic solution is prevented by previous hemorrhage. Blood volume figures indicate that the liquid interchange is about as in unbled animals. The question of the departure of the behavior of the solids in this animal from the behavior of the interchanges of solids in animals without previous hemorrhage will be considered below. In the case of dog 9 the 15 per cent depletion of the blood is more than made up by the time of the termination of the injection (blood volume raised to 106 per cent of normal); this volume falls in 2 hours to 97 per cent, then very slowly to reach 93 per cent at the end of 7 hours. The gum acacia is in all probability filling the place of the removed plasma proteins in holding water.

2. Blood composition. The main point of interest is, What is the nature of the fluid drawn into the blood stream and how does its composition change subsequently? A satisfactory answer to these questions should serve to elucidate the processes determining the interchanges.

a. Protein. Any conclusion as to the passage of protein into or out of the blood stream must be based on calculations of the absolute amount of plasma protein in the entire blood stream. If, for instance, it should be found that the curve representing the percentage of plasma protein ran parallel to the curve representing the reciprocal of blood volume, this would not mean that the absolute protein content of the plasma was unchanged, since practically all of the fluid drawn into the blood stream enters the plasma and an increase of 15 per cent in blood volume would mean an increase of over 23 per cent in plasma volume. The

method used here of calculating absolute amounts of plasma protein is essentially that used by Magnus (10), some changes in the method being introduced in order to allow for the hemoglobin withdrawn in the samples. It assumes that the blood is 9 per cent of the body weight (24), (25), (26), this figure being used instead of 7 per cent used by Magnus, that the blood is 64 per cent plasma by volume and that all the water drawn into the blood stream enters the plasma. While none of these three assumptions is strictly correct, the first and third are very nearly so and variations in the second will not involve gross errors since the calculations for the additional samples of any one animal are based upon the plasma volume figure obtained in the calculation of the standard blood of that animal. A sample calculation may be given for purposes of illustration.

Sample calculation. Dog 7. Body weight, 9.1 kilos

HEMOGLOBIN				TOTAL NITROGEN*	
Sample number	Reading	Volume per cent uncorrected	Volume per cent corrected	Sample number	Grams N per 100 cc.
1	20.0	100	100	1	0.83
2	22.6	113	111	2	0.74
3	22.0	110	107	3	0.76
4	21.4	107	102	4	0.77
5	21.0	105	98	5	0.80

* The terms "N" or "Total N" refer to protein N, i.e., the non-protein N has been subtracted from the Kjeldahl figure.

Method of correcting blood volume for hemoglobin withdrawn. For each sample 13 cc. of blood are removed. This is 1.6 per cent of the blood volume of this animal, or 1.6 per cent of 819, which is 0.09×9100. Since, after the first sample, only 98.4 per cent of the original amount of hemoglobin remains in the blood stream the increase in volume of the blood is only 98.4 per cent of that indicated by the blood's dilution as shown by the hemoglobin readings in the colorimeter. To illustrate, assume that 5 per cent of blood and therefore of hemoglobin is withdrawn from a normal dog, assume that the normal or standard sample reads 20 in the colorimeter, as in our case, and assume further that a second sample drawn subsequent to the withdrawal of 5 per cent blood volume reads 21 in the colorimeter. If we failed to consider the previous loss of the 5 per cent hemoglobin we would infer from this reading that the blood volume had increased 5 per cent, was now 105 per cent of the normal. What has actually happened, however, is that the blood volume has been replaced just to its original state, the diluent being of course hemoglobin-free, so that each unit volume of blood now contains only 95 per cent of its normal amount of hemoglobin. To find the true blood volume we should take 95 per cent of 105 or 100 per cent,

in round numbers. To find the true blood volume in any case, therefore, we should multiply the apparent blood volume by the percentage figure of hemoglobin remaining in the blood stream at the time of drawing the sample.

Upon the basis of this treatment of the data we find that in dog 7 the blood volume at the time of drawing the last sample is actually slightly decreased rather than increased, as the hemoglobin readings would seem to indicate, i.e., the decrease in the percentage of hemoglobin in the last sample is not great enough to account for all the hemoglobin removed. We must assume, therefore, that the blood volume has fallen slightly. The decrease, however, is not as great as the volume lost by hemorrhage. This means that some of the fluid drawn in has stayed in but not quite enough to equal the amount lost by hemorrhage. In all the other normal animals the final blood volume even after correction for the withdrawal of blood was slightly above the initial or normal volume.

Using the corrected blood volume figures we may proceed with the *method of calculating total plasma nitrogen*. It may be noted here that the figures for blood volume per cent given in all the tables have been corrected for the amount of blood lost according to the method outlined above.

$0.09 \times 9100 = 819$ cc., blood volume at beginning, blood 1.

$0.64 \times 819 = 524$ cc., plasma 1.

$0.83 \times 5.24 = 4.35$ grams N in plasma 1.

Blood 2 is 111 per cent of blood 1, by volume. The 111 per cent increase has been in the plasma. $0.11 \times 819 = 90$ cc. increase.

Plasma 1 = 524 cc. $524 + 90 = 614$ cc., plasma 2.

$0.74 \times 6.14 = 4.54$ grams N in plasma 2.

Blood 3 is 107 per cent of blood 1. The 7 per cent increase has been in the plasma. $0.07 \times 819 = 57$ cc. $524 + 57 = 581$ cc., plasma 3.

$0.76 \times 5.81 = 4.42$ grams N in plasma 3.

By the same method we get $0.77 \times 5.40 = 4.16$ grams N in plasma 4.

$0.80 \times 5.08 = 4.06$ grams N in plasma 5.

These figures indicate that by the time sample 5 was drawn protein had passed out of the blood stream, i.e., less protein is in the plasma than was there at the beginning. If, however, we add to each plasma N figure the amount of plasma N taken out in that sample and in preceding samples we get the following figures: The amount of N withdrawn with each sample is $0.64 \times 13 \times 0.008 = 0.07$ gm. N, 0.008 being taken as the average amount of N per cc. plasma in this animal. Variations in different samples are not significant in calculations on 13 cc. The effect is of course cumulative; 0.14 gm. will have been removed by the time the third sample is drawn, 0.21 gm. by the time of the fourth, etc.

SAMPLE NUMBER	PROTEIN N		
	N remaining	N withdrawn	Total protein N
	grams	*gram*	*grams*
1	4.35	0.0	4.35
2	4.54	0.07	4.61
3	4.42	0.14	4.56
4	4.16	0.21	4.37
5	4.06	0.28	4.34

These figures show that there is at first a slight increase in the amount of plasma protein but that by the time of the fifth sample this has disappeared, and that the final total plasma protein N is the same as the original figure if the amount withdrawn is added. Different animals show some variations from this behavior.

The figures obtained on the dogs without hemorrhage are not constant. All show that there is a marked decrease in the concentration of plasma protein accompanying the increased blood volume; but two cases, dogs 3 and 7, show that this decrease is not quite so great as the increase in plasma volume, i.e., a slight amount of protein enters the blood stream; while two others, dogs 4 and 6, indicate that a slight amount of protein passes out of the blood. Of these cases, however, the figures for dog 7 are the most reliable, since they were obtained by Kjeldahl determinations, using 2 cc. of plasma, while the others were obtained by indirect Nesslerization, working with 0.05 cc. of plasma. While the figures are not convincing, it appears that there is a slight increase in the absolute amount of plasma protein immediately after the injections. If the amounts of protein withdrawn in taking the samples are added to the amounts remaining we find a greater, though still quite slight, increase and that the amount of plasma protein tends to become constant in most cases at a level slightly above or about the same as the original one.

Scott (27) showed that dilution of the blood *in vitro* with isotonic Ringer's solution causes protein to pass from the blood cells into the plasma. He uses this observation to account for the increase in total plasma protein following hemorrhage or the injection of isotonic Ringer's solution (28). By inferring that slight variations from the proper proportions of the salts in the fluid added to the blood greatly inhibit this transfer of protein, he accounts for the greater increase in plasma protein after hemorrhage.

In order to determine whether or not the increase in total plasma protein which we found could be due to a passage of protein from blood cells to plasma the following experiment was performed. From an 8 kilo dog 140 cc. of blood were drawn into a vessel containing the minimum amount, 0.12 gm. per 100 cc. blood, of potassium oxalate that would prevent clotting. This was stirred and 45 cc. samples immediately put into each of three cylinders. All three were stirred continuously while to one was added 2.5 cc. of the gum-glucose solution at a constant rate over 40 minutes, to the second 2.5 cc. of gum-glucose solution + 5 cc. Ringer's solution with the calcium omitted; the third was merely stirred and used as a control. The second of the above mentioned procedures may be assumed to approximate the conditions obtaining in the intravenous injection *in vivo* of 5 cc. of the gum-glucose solution per kilo body weight or per 90 cc. blood, the 5 cc. Ringer's per 45 cc. blood being the average increase in blood volume over the amount of fluid injected. After the addition of the solutions was completed hematocrit determinations were made on each sample and Kjeldahl nitrogen determinations made on each plasma.

SAMPLE	HEMATOCRIT			PLASMA IN 45 CC. BLOOD	PROTEIN IN PLASMA
	Total	Cells	Plasma		
				cc.	*gram*
1 (45 cc. blood)	10.7	4.2	6.5	27.4	0.259
2 (45 cc. blood + 2.5 cc. g.g.)	11.0	4.1	6.9	28.2	0.260
3 (45 cc. blood + 2.5 cc. g.g. + 5 cc. Ringer's)	11.4	3.6	7.8	30.8	0.257

The results collected in the accompanying table show that there was no exchange of protein between cells and plasma. It seems fair, therefore, to eliminate the blood cells as a source of the increased plasma protein in our *in vivo* experiments.

Kjeldahl determinations were also made on the gum-glucose used in order to determine if it might be a source of N. It was found that 2 cc. of the solution is equivalent to 0.6 cc. tenth normal HCl. Expressed in percentage of N our determination shows 0.168 per cent N in the gum acacia or 0.042 per cent N in the solution injected. Rideal (29) finds gum acacia to contain only 0.031 to 0.082 per cent N. Other observers have reported varying percentages of N, depending upon the source of the gum. The form in which the N exists has never been determined, so far as we have been able to learn. The amount of the solution used

for a 10 kilo dog, 50 cc., would contain only 0.021 gm. N, according to our determinations. Corpuscles and injected solution may therefore be disregarded as a source of the increase in plasma N. Our results lead us to the conclusion, therefore, that the injection of the acacia-glucose solution leads in some way to the entrance of protein into the circulation.

The changes in the percentage concentration of plasma protein following the injection are also of interest in that they indicate roughly the changes in the colloidal osmotic tension of the blood. It is seen that the concentration falls markedly immediately after the injection, considerably more than can be accounted for by the blood's dilution due to the injected fluid alone. Let us assume that each 5 cc. of the injected solution contains 4.5 cc. of water. Since 4.5 cc. of water are injected per kilo body weight or per 90 cc. of blood or per 58 cc. of plasma, the plasma is diluted 7.8 per cent by the water injected. The plasma protein percentage, however, invariably falls more than this, averaging about 15 per cent, showing that not only the fluid injected but the fluid drawn in from the tissues dilutes the plasma in respect to its protein content. The plasma protein content in the subsequent hours rises toward normal but never reaches it, this state of affairs co-existing with a slight persisting increase in blood volume. The assumption seems justified that part of the colloidal osmotic tension of the plasma is being supplied by the injected gum acacia.

Magnus reports two experiments with intravenous injection of concentrated (35 per cent) NaCl solution. In one case he finds the total plasma proteins diminished by 7 per cent by this procedure, in the other case increased by 5 per cent. These were on previously unbled dogs and the results are comparable to our results on previously unbled dogs.

Dog 8, *which was bled 15 per cent of his blood volume* before the injection, shows a decrease in total plasma protein at the second sample. When, however, the amount of plasma protein withdrawn by hemorrhage is added to the total amount in the blood at the time the second sample was drawn we find a slight increase in the plasma protein—some protein has passed in.

b. Sugar. It is seen that the initial blood sugar values in the first 8 cases are very high, 0.192 to 0.308 gm. per 100 cc. This is due to the well known action of morphine as a respiratory depressant, the imperfect respiration causing a hyperglycemia. At the close of the injection the figure is still higher, 0.247 to 0.564 gm. per 100 cc., due to the in-

jected glucose. Within 2 hours the figure has usually fallen to or below the initial value. The sugar figures on dog 9 are much the most valuable since here the disturbing effect of the morphine is not present. The initial figure is 0.133, the slight increase above normal probably being due to the ether; the figure immediately at the termination of the injection is 0.292; 2 hours later, 0.163; 2 hours later, 0.133; and the last sample, 2 hours after this, contains 0.135 gm. These figures show that the sugar rapidly passes out of the blood, and is almost back to normal in 2 hours. The fate of the sugar will be considered later.

Since the increased blood volume persists for several hours after the blood sugar has returned to normal it must be concluded that gum acacia remains in the circulation and holds water there. The great difficulty of digestion for nitrogen determinations of all the samples taken subsequent to the injection, even those which had regained a normal blood sugar value, indicates that there was an abnormally large amount of carbonaceous material in the blood and confirms the view that gum acacia remains in the blood for some time.

c. Plasma chlorides. The plasma chlorides were followed because it was felt that their behavior under the conditions of the experiment would be typical of that of the inorganic crystalloids in general. The percentage concentration of plasma chlorides always falls in the second sample, but not in any way to the same extent that the blood is diluted; in fact, the percentage fall in concentration is not even as great as the percentage of dilution of plasma accomplished by the injected fluid. It has been seen above that the plasma is diluted about 7.8 per cent by the injected fluid. The percentage fall of plasma chloride concentration is less than this, averaging about 6 per cent. In other words, the fluid drawn into the blood stream carries with it chlorides in concentration equal to that of plasma and in addition to this inward filtration of chlorides an inward diffusion has started to supply chlorides for the injected fluid. This diffusion continues for several hours, the percentage of plasma chlorides steadily rising and becoming normal in 6 to 8 hours.

d. Non-protein nitrogen. It will be noted that the plasma N.P.N. concentration remains constant with variations in plasma volume, indicating that the vessel walls are more permeable to urea than to NaCl. The constant concentration of N.P.N. means that not only does the fluid drawn in have a N.P.N. concentration equal to that of plasma but also that the diffusion into the blood stream of the N.P.N. necessary to make up the injected fluid to the concentration of N.P.N. existing in plasma is very rapid, being completed by the time of completion of the

injection. Urea's greater solubility in lipoids, which are assumed to be present in cell walls, may account for this. An alternative view is that the urea does not diffuse into the blood stream but is secreted into the blood stream at the proper rate to maintain a constant concentration. Our methods and data do not afford us any means of deciding which of these processes actually occurs but the former seems the more probable.

e. Rather rough estimations of the *crystalloid osmotic tension* of the plasma samples, using the method of calculation mentioned above, show that while there may be some attempt on the part of the organism to keep this tension constant, the compensatory mechanism, if such exists, is not adequate to keep pace with the factors tending to vary the osmotic tension.

3. Urine. When morphine had been given sugar always appeared in the urine in rather high percentage, 5 to 6 per cent. In two cases, dogs 6 and 8, a marked diuresis resulted; in two others, dogs 5 and 7, urinary secretion was practically normal in volume. Dog 9, which had no morphine, showed only a trace of sugar and his volume excretion was normal or slightly increased. As to the fate of the injected glucose, it is evident that it does not stay in the blood and that only a small fraction is excreted in the urine, in fact only a trace when morphine is omitted. It will be noted that the injection is so timed that the anesthetized animals receive glucose at the rate of 0.9 gm. per kilo per hour, the unanesthetized dog's tolerance rate (11). No study of the tissue fluids or glycogen depots was made but we may conclude with Brasol that the remainder is "perhaps changed to glycogen, lactic acid or undergoes some other chemical metamorphosis." The observation of Fisher and Wishart (9) that the metabolism is increased 20 per cent accounts for a part of it.

It is evident, confirming the finding of Meek and Gasser (22), that the gum acacia injected with the glucose does not diminish urinary secretion, as is maintained by Kruse (30); in fact in the cases where hyperglycemia was extreme, with blood sugar values around 0.500 gm. per 100 cc., glycosuria occurred with an accompanying diuresis. Two cases with hyperglycemia and glycosuria, dogs 5 and 7, while not exhibiting a diuresis, certainly did not show a suppression of urine. The urine was not followed in the first four cases. In the case of dog 9, where morphine was omitted with the result that hyperglycemia was not so marked and no significant glycosuria occurred, excretion rate of water was normal or slightly increased. Knowlton (31) finds that colloids exerting osmotic pressure, such as gum acacia, inhibit NaCl

diuresis but have but little effect on Na_2SO_4 diuresis. Glucose diuresis belongs to the same class as that of NaCl, i.e., it is presumably due to purely mechanical factors, such as hydremic plethora, rather than to a direct action on the kidney cells. We might explain, then, the normal excretion of urine following the injection of the gum-glucose solution, in the absence of morphine, as due to a balance arrived at between the inhibitory action of the acacia and the diuretic action of the glucose, the later gaining the upper hand when, due to morphine, a marked hyperglycemia is produced with a resultant glycosuria.

SERIES II. ASPHYXIATED DOGS

There is evidence that asphyxia increases the permeability of the vessel walls. Bolton (32) holds that permeability is increased by stagnation of blood with decreased O_2 supply, since edema of the neck is produced by ligature of the superior vena cava, despite the absence of any rise in pressure in the jugulars and presumably in the capillaries. Starling (33) also draws the conclusion that the edema under these conditions must be due to increased permeability of walls which allows protein to pass out into the tissues and hold water there.

The present experiments on asphyxiated animals were carried out with these statements in mind. Study of the data (table 2) shows that, owing probably to the asphyxia, the blood sugar figures are very high, 0.133 and 0.244 before and 0.800 and 0.770 immediately after the injection. In both cases the value 2 hours after the injection was below the initial value, the asphyxia having long since worn off. Except for this no significant departure from the behavior in normal animals occurs. The asphyxia introduces so many complicating circulatory factors, however, that we are not justified in concluding that the permeability of the vessel walls is unchanged, although we have no proof that it is. For example, protein might have been drawn in but promptly squeezed out again due to the asphyxial rise in blood pressure. It will be noted that even without morphine here the hyperglycemia is extreme and the glycosuria marked but that the blood sugar rapidly falls to or below the normal. The apparent continuance of the glycosuria after the blood sugar has fallen to normal is probably due to the fact that the bladder was not completely emptied at each catheterization, urine containing sugar which had really been excreted during the period of hyperglycemia being obtained at each sample.

TABLE 2

Data of series 2, asphyxiated dogs

DOG	SAMPLE	TIME	BLOOD VOLUME	PLASMA CHLOR.	SUGAR	N. P. N.	PROTEIN N				URINE		
							Remaining	Withdrawn	Total	Per 100 cc. plasma	Amount	Sugar	Sugar
		hours	*per cent*	*grams per 100 cc.*	*grams per 100 cc.*	*mgms. per 100 cc.*	*grams*	*grams*	*grams*	*grams*	*cc.*	*per cent*	*grams*
10	1		100	0.635	0.133	25	5.25		5.25	0.980	20	0	0
	2	0	111	0.585	0.800	25	5.55	0.08	5.63	0.881	14	6.4	0.9
	3	2	108	0.625	0.118	26	5.57	0.16	5.73	0.920	40	5.8	2.3
	4	4 35′	105	0.620	0.111	25	5.27	0.24	5.51	0.914	6	5.2	0.3
	5	8	102	0.630	0.182	25	5.15	0.32	5.47	0.930	75	3.4	2.6
Total after injection											135		6.1
11	1		100	0.650	0.244	28	4.77		4.77	0.902	160	0	0
	2	0	116	0.592	0.770	29	4.95	0.07	5.02	0.742	18	+	
	3	2 5′	114	0.640	0.171	28	4.94	0.14	5.08	0.761	12	+	
	4	4 25′	110	0.636	0.167	29	4.74	0.21	4.95	0.770	20	+	
	5	6 45′	102	0.642	0.164	28	4.76	0.28	5.04	0.870	135	+	
Total after injection											185		10.1

TABLE 3

Data of series 3, shocked dogs

DOG	SAMPLE	TIME	BLOOD VOLUME	PLASMA CHLOR.	SUGAR	TOTAL N				URINE		
						Remaining	Withdrawn	Total	Per 100 cc. plasma	Amount	Sugar	Sugar
		hours	*per cent*	*grams per 100 cc.*	*grams per 100 cc.*	*grams*	*grams*	*grams*	*grams*	*cc.*	*per cent*	*grams*
12	1		100	0.632		8.30		8.30	1.004			
	2		88	0.642		6.67	0.08	6.75	0.993			
	3		127	0.592		7.54	0.16	7.70	0.770			
13	1		100	0.629	0.154	10.15		10.15	0.976			
	2		84	0.633	0.145	7.64	0.08	7.72	0.980	24	0	0
	3		129	0.550	0.404	8.32	0.16	8.48	0.707	18	0	0
	4	1 52′	124	0.580	0.154	9.16	0.24	9.40	0.826	26	0	0
Total										68		
15	1		100	0.635	0.200	5.74		5.74	0.818	140	0	0
	2		84	0.645	0.220	4.16	0.06	4.22	0.791			
	3		117	0.594	0.444	4.45	0.12	4.57	0.651	10	3.0	0.3
	4	2 10′	114	0.610	0.274	4.40	0.18	4.58	0.672	34	4.2	1.4
	5	4 35′	111	0.640	0.172	4.30	0.24	4.54	0.686	2	4.0	0.08
	6	6 45″	107	0.644	0.141	4.27	0.30	4.57	0.723	0		0
Total after injection										46		1.8

SERIES III. SHOCKED DOGS

Dog 12. Body weight, 14.36 kgm. No morphine. Specimens from pancreas, liver, spleen, large and small intestine and kidney for histological study. B. P. at 9:55 is 160 mm. First sample at 10:10, B. P. then 150. Cava clamped at 10:25, B. P. immediately fell to 40 mm. 11:30, B. P. 40, pulse 120, very little ether required. 12:40, B. P. 40, clamp removed, B. P. rose slowly, reached 50 in 3 minutes, 55 in 6 minutes. Second sample, at 12:52, B. P. fell sharply to 38; injection started at 12:57. B. P. soon fell to 34, respirations of expiratory type, heart very irregular. As injection proceeded respiration and heart action improved, B. P. climbed to 40 in 15 minutes. 1:25, B. P. 60. 1:30, animal tried to vomit, intestines extruded from wound, B. P. fell to 40, rose again to 54 in 5 minutes. 1:50, B. P. 60. 1:55, B. P. 65. 1:57, injection ended. 2:03, 3rd sample. Animal tried to vomit during taking of sample 3, B. P. fell to 42. 2:10, B. P. 34. 2:13, animal died. It will be noted that in this severe grade of shock the loss of 13 cc. of blood almost killed the dog (sample 2) and an additional loss of 13 cc. did produce death (sample 3).

Dog 13. Body weight, 18.05 kgm. 1:00 p.m., B. P. 125. 1:20, B. P. 128, 1st sample drawn. 1:25, cava clamped, B. P. fell to 40 and was maintained there. 1:30, ether discontinued. 3:30, clamp removed, B. P. rose to 60 in 2 minutes, amplitude of fluctuations very great. In 4 minutes B. P. averaged 80. 4:00, 2nd sample, B. P. 100, not affected by drawing 13 cc. blood. Injection started at 4:08, 4:20, B. P. 110. 4:30, B. P. 115, ether required. 4:40, B. P. 120. 4:43, B. P. 125. 5:03, 130. 5:08, injection ended. 5:12, 3rd sample, 5:15, B. P. 110, ether almost continuously since 4:30. 5:50, B. P. 90. 6:00, B. P. 80. 6:10, B. P. 64, pulse regular, ether discontinued. 6:25, B. P. 58. 6:40, B. P. 50. 6:55, B. P. 40. 6:57, B. P. 30. 6:59, 4th sample, animal died immediately. This case illustrates well the type of shock in which the blood pressure is well maintained for some time, masking the true severity of the shock; eventually comes the rather sudden terminal circulatory and respiratory collapse.

Dog 15. Body weight, 12.2 kgm. 10:10, 1st sample. 10:20, B. P. 140. 10:25, cava clamped, B. P. fell to 40 and was maintained there. 12:25, clamp removed. B. P. rose to 100 on removing clamp. 12:30, 2nd sample, B. P. 110. 12:35, injection started, B. P. 110. 12:50, B. P. 130. 1:25, B. P. 125. 1:35, injection ended. 1:37, 3rd sample, B. P. not affected by drawing sample. 2:30, B. P. 110. 3:35, B. P. 100. 3:45, 4th sample, B. P. 90. 6:10, 5th sample, B. P. immediately before sample = 80, immediately after = 65. 6:25, B. P. 60. 6:40, B. P. 55. 7:15, B. P. 45. 8:20, 6th sample, B. P. 35 immediately before, dropped to 30 immediately after, death in few minutes.

It is now believed practically universally that in traumatic shock blood plasma leaves the circulation. Inasmuch as the concentration of the proteins of the plasma remaining in the vessels is not appreciably changed it must be assumed that in shock the permeability of the vessel walls is increased. The present series of experiments was planned in order to ascertain whether the fluids drawn into the blood stream by the hypertonic solution in fatally shocked animals would carry along

with them more protein than this procedure brings in when applied to normal animals. Four animals were used, but one died in shock before the injection was completed. The results from the other three are given (table 3). Non-protein nitrogen was not followed in these cases as blood was so valuable and it was felt that this determination could best be omitted. The only respects in which the results of this series differ from those of the normal series are in blood volume and protein content.

1. Blood volume. In accordance with the results of numerous previous observers we find the blood volume greatly diminished in shock. In this series the second or "shocked" sample was taken as the standard for subsequent determinations of hemoglobin and blood volume. As a result of the injection of the gum-glucose solution the blood volume is markedly increased above its shock level, the increase even bringing the blood volume above its initial normal level in all three cases. The volume then gradually falls off until (in the one case which could be followed for several hours, dog 15) at the end of 7 hours after the injection it reaches approximately its initial normal level. This was shortly before the animal died.

2. Plasma protein. The method of calculating the absolute amounts of plasma protein may be illustrated here. The case of dog 12 may be taken.

Body weight 14,360 grams

HEMOGLOBIN*				TOTAL NITROGEN†	
Sample	Reading	Volume per cent uncorrected	Volume per cent corrected	Sample	N per 100 cc. plasma
					grams
1	20.0	100	100	1	1.004
2	17.8	89	88	2	0.993
3	25.6	128	127	3	0.77

* Sample 1 is taken as the standard for reading sample 2, sample 2 as the standard for reading sample 3 and any subsequent samples that may be obtained.

† Since N.P.N. was not followed in shocked animals the figures given here as "total N" are the actual Kjeldahl figures. Practically no change in the relations of the figures is produced by the subtraction of the small constant N.P.N. figure from each total N figure, as was done in the first two series.

Calculation. $0.09 \times 14{,}360 = 1292$ cc., blood 1.
$0.64 \times 1292 = 827$ cc., plasma 1.
$1.004 \times 8.27 = 8.30$ gm. N. in plasma 1.
Blood 2 has 88 per cent volume of blood 1, i.e. 12 per cent less.

Assume this has all been taken from the plasma. This probably is not correct because of accumulation of corpuscles in the periphery. This peripheral aggregation of corpuscles, however, would, as is mentioned below, make the calculated exchange of protein even greater if it could be quantitatively considered.

12 per cent of 1292 = 155. 827 − 155 = 672 cc., plasma 2.

6.72 × 0.993 = 6.67 gm. N in plasma 2.

Volume of blood 2 is 88 per cent of blood 1, i.e., 1137 cc.

Volume of blood 3 is 127 per cent that of blood 2.

The extra 27 per cent of 1137 or 307 cc. has entered the plasma.

Plasma 2 = 672 cc. 672 + 307 = 979 cc., plasma 3.

9.79 × 0.77 = 7.54 gm. N in plasma 3. Summarizing in the form of a table we have:

SAMPLE NUMBER	PLASMA N REMAINING	PLASMA N WITHDRAWN	TOTAL PLASMA N
	grams	*gram*	*grams*
1	8.30		8.30
2	6.67	0.08	6.75
3	7.54	0.16	7.70

We find that the absolute amount of plasma protein is greatly diminished in shock, its concentration remaining practically constant or being very slightly diminished. This is in agreement with the refractometric findings of Gasser, Erlanger and Meek (34). With the increase in blood volume determined by the injection the absolute amount of protein rises markedly above its shock level but does not reach the normal level. The concentration falls considerably but not to the same degree as plasma volume is increased. In other words, the fluid drawn in is not so rich in protein as is plasma but neither is it protein-free. Due to the method of following the blood volume these figures probably do not indicate the real magnitude of the changes in total protein. As is well known, the slowed circulation of shock causes red blood cells to accumulate in the capillary area so that the number in the arterial blood, in which the hemoglobin was followed, is relatively small and the estimated blood volume therefore high. And it is to be presumed that the improved circulation following the injection of the gum-glucose solution will return some of the jammed corpuscles to the circulation, causing the blood volume estimation to be too low.

Following the blood changes after the injection, it is found that protein continues to increase even while water is passing back out, the protein concentration rising faster than the plasma volume falls. What may happen is that when the injection is given the fluid drawn in brings

with it protein through the abnormally permeable walls, but in lower concentration than it occurs in plasma. Then, as the blood pressure rises, the circulation improves and lymph flow is reëstablished. The lymph flow from the liver and intestines is probably accelerated to a far greater degree than that from the extremities, for two reasons: *a*, In these animals the circulation of both posterior extremities was practically done away with since both femoral arteries and veins were ligated. *b*, Since the capillaries of the liver and intestines are the most permeable it is probable that most of the protein that disappeared from the plasma in the process of the development of shock passed out into the tissue fluids of the liver and intestines. As the circulation to these organs is now improved the normal lymph flow is reëstablished and this lymph flow sweeps along with it back into the blood stream through the thoracic duct the plasma protein which had accumulated in the tissue spaces of the liver and intestines during the induction of shock. Thus protein is entering the blood stream even while blood volume is falling. Such an interpretation of the results accounts for the rapid initial increase in blood volume with an increase in absolute amount, although a decreased percentage, of plasma protein, for the subsequent falling off in volume and for the concomitant continued increase in the absolute amount of protein. The prospect of technical difficulties in collecting lymph over a long period of time has kept us from making direct observations on this latter point.

In the shocked animals, as in the normal and asphyxiated, the final sample shows a percentage concentration of plasma protein lower than that of the initial sample and at the same time the blood volume at the time of the final sample is greater than initially; or, as in the case of dog 15, who was followed for 8 hours, when the blood volume has in time fallen to slightly below its initial normal level, the percentage concentration of plasma protein has fallen to a considerably greater extent. This must mean that here too gum acacia is taking the place of plasma protein in holding water in the circulation.

Starling (35) says "Absorption by the blood vessels as a result, say of artificial hemorrhage, if determined entirely by the osmotic attraction of the plasma colloids for the extravascular fluids, can only bring about a passage of water and salts into the blood vessels. . . . According to my explanation this (absorbed) fluid should be pure salt solution. That it is more dilute than plasma is clearly shown by experiments but our data do not yet suffice to determine whether the incoming fluid is a weak solution of protein, such as that contained in the tissue

spaces, or is a pure salt solution. If it is proved by quantitative results to contain protein, then some other factor, such as back filtration or active absorption by the endothelial cells of the blood vessels, must be involved in addition to the colloid constituents of the circulating blood." The present data point strongly to the conclusion that some protein does pass in directly in the case of shock although it might not be impossible to explain all of our data on the assumption that the protein is carried back into the blood stream by the lymph. The rapidity with which it occurs, however, speaks against this.

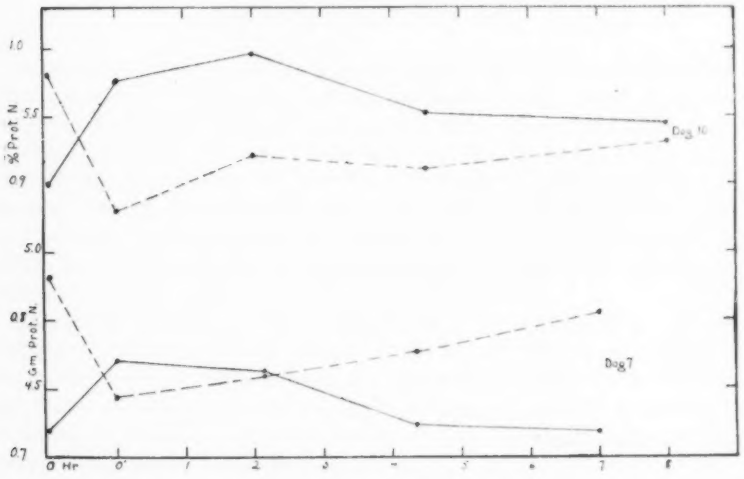

Fig. 1. Changes in total amount (solid line) and per cent (broken line) of plasma protein N in a normal animal, dog 7, and in an asphyxiated animal, dog 10. Time of taking 1st sample is indicated by *0*, the end of the injection by *0'*.

3. Urine. The urine in the case of dog 13, a shocked animal, contains no sugar in spite of the hyperglycemia which far exceeds the normal overflow threshold. This dog was in rather severe shock and the absence of glycosuria is probably due to the fact that no urine was secreted after shock developed. The fact that additional samples of urine could be drawn probably means that the bladder had not been completely emptied of its pre-shock urine. Dog 15 was in better condition and excreted urine containing sugar.

4. Non-toxicity of the acacia-glucose solution. It was our intention in these experiments to produce a grade of shock that would prove fatal in a few hours and in this we succeeded. Some of the animals were almost moribund when the solution was administered. In no case

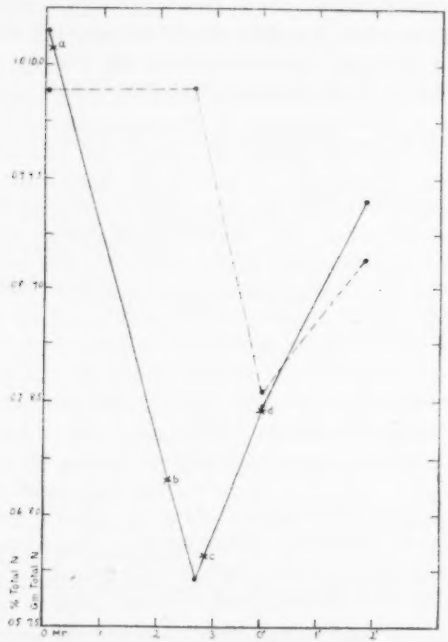

Fig. 2. Changes in total amount (solid line) and per cent (broken line) of plasma total N in a shocked animal, dog 13. Letters designate times of procedures, as follows: *a*—cava clamped; *b*—clamp removed; *c*—injection started; *d*—injection ended. Time of taking 1st sample is designated by *0*, while *1*, *2*, etc., designate hours after taking 1st sample up until end of injection. The end of the injection, when the 3rd sample is taken, is designated by *0'*, while *1'*, *2'*, etc., designate hours after end of injection.

were any untoward effects observed as a result of the injection; the blood pressure, heart action and respiration always were benefited. The rectal temperature was practically unchanged. In one case a slight albuminuria was observed in one sample, but microscopic exami-

nation revealed large numbers of spermatozoa. The albuminuria had disappeared in the next sample. Casts, hematuria or hemaglobinuria were never observed. No hemolysis occurred as a result of the injections, some slight laking being present in several plasma samples but it was no more evident in samples taken after the injection than in those taken before. This laking is attributed to the ether. These findings in regard to the non-toxicity of gum acacia are in accord with those of Bayliss (36), who discusses the rather misleading statements of Kruse (30) as to the toxic effects observed after the injection of acacia.

SUMMARY AND CONCLUSIONS

A strongly hypertonic glucose and gum acacia solution was injected intravenously into normal, asphyxiated and shocked dogs, and the resultant changes in blood volume and composition were studied.

The immediate effect was a marked increase in blood volume; in normal and asphyxiated animals the blood volume then gradually fell toward but did not completely return to normal in several hours.

The blood volume, markedly diminished in shock, is increased to above its normal level by the injection and then gradually falls to or below its normal level.

The absolute plasma protein is increased slightly or not at all in normal animals and in asphyxiated animals; in an animal which had been bled there was a slight increase when the amount withdrawn was allowed for. The absolute amount of plasma protein is markedly diminished in shock, is increased by the injection and the increase continues for some time after the injection. It is believed that at least a part of the increase in plasma protein following the injection in shock is due to a passage of protein in through the vessel walls.

Gum acacia seems to take the place of plasma protein in holding water in the circulation.

There is a marked hyperglycemia immediately after the injection in normal animals; this is accentuated by morphine and asphyxia. The blood sugar value falls to or nearly to normal within 2 hours. In shocked animals the blood sugar behaves much as in normal animals. There is only a trace of sugar excreted by normal animals excepting when morphine or asphyxia cause marked glycosuria. Shocked animals without morphine excrete some sugar unless, as a result of the shock, there is a suppression of urine.

The fluid drawn into the blood stream brings with it chlorides in concentration equal to the chloride concentration of plasma but the diffusion into the blood stream of sufficient additional chlorides to bring the chloride concentration of injected fluid up to that of plasma is not complete for several hours.

The entrance of urea into the plasma takes place with such facility that the non-protein nitrogen concentration of the plasma remains constant.

There is no suppression of urine in normal animals as a result of the injection, if anything the rate of secretion is slightly increased.

The crystalloid osmotic tension of the plasma does not remain constant.

No hemolysis, hematuria, hemoglobinuria, albuminuria, cylindruria, fluctuations in body temperature or any other untoward effects were observed as a result of the injections.

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THE FUNCTIONAL ACTIVITY OF THE CAPILLARIES AND VENULES

D. R. HOOKER

From the Physiological Laboratory of the Johns Hopkins University

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INTRODUCTION

The significance of the blood stream in the capillary bed for the nutritive processes of the body is well recognized. But it is only in recent times that emphasis has been laid on the capillaries as a factor in the dynamics of the circulation. It is the purpose of this paper to present evidence in support of the belief that the capillaries and venules, as well as the arterioles, respond to direct chemical stimulation and also to indirect stimulation through the intervention of nerve fibers. If this proof is established, the functional activity of the capillary and venous beds must henceforth increase in both theoretical and practical importance. In the first place local tissue needs may by direct chemical action control the capillary blood streaming through the part as shown so clearly by Krogh (1) for muscle. In the second place, the capillaries and venous fields being under the influence of the central nervous system, it follows that local vascular reflexes (2) and systemic vascular reflexes undoubtedly depend upon the coöperation of the capillary and venule with the arteriole; that, in other words, the peripheral resistance, both functional and static, includes arteriole, capillary and venule. And in the third place the effective blood volume, both fluid and corpuscular, must be subject to alteration and regulation to a very significant degree.

Most of the work on the function of the capillaries hitherto reported, except the recent paper by Krogh just mentioned, has been done on the frog in which the transparency of the tissues permits microscopic visualization of these vessels. It is possible, however, using Lombard's method of a drop of oil on the skin (3) to extend such studies to the mammal. Furthermore, most of the recent evidence in the mammal bears upon dilatation of capillaries. It may be assumed that a vessel

which is shown to dilate must also contract, but the direct evidence has hitherto been lacking. Nor is there much evidence that the blood capillary and the venule are under nervous control.

HISTORICAL

The first evidence that capillaries have the power independently to change their caliber was published in 1858. In that year Lister (4), describing the early stages of inflammation, pictured with camera lucida drawings a very great increase in the caliber of the capillaries in inflamed tissue. This observation was followed by the studies of Stricker published some seven years later (5). Stricker observed the capillaries in the nictitating membrane of the frog to dilate and to constrict. The constriction was less evident than the dilatation and appeared to be due to two processes, one a nuclear swelling and the other an actual contraction of the endothelial protoplasm. These results were obtained in tissue removed from the body and therefore deprived of its blood supply, and Stricker himself is not convinced that they are not due to lethal changes in the tissue. This possibility is supported by figure 4 in his paper, which pictures a change in shape of a capillary vessel which must be extremely unusual if compatible with functional activity. In a second paper published the following year Stricker (6) describes further experiments with the nictitating membrane of the frog removed from the body and examined in the aqueous humor under the microscope. Many of these preparations were entirely unsatisfactory but a few of them gave beautiful responses to stimulation by ammonia vapor. When the tissue was exposed to ammonia vapor for three or four seconds and then examined under the microscope, Stricker saw the capillary lumina almost disappear and then dilate wide enough for a corpuscle to pass. This phenomenon occurred about twice in fifteen minutes. Further, Stricker observed a varicosity on a capillary which moved forward, suggesting peristaltic activity on the part of the endothelial tube. When the capillaries thus under observation contracted they became practically invisible but could be readily seen again after the contraction had passed off. Stricker likewise employed electrical stimulation. The procedure which he used is not clear but with it he obtained repeatedly good results on various specimens. When stimulated, the capillaries would contract almost instantaneously and dilatation would follow a moment or more after the cessation of the stimulus. After a few applications of the stim-

ulus no further response could be obtained. Even better results were had in the case of the capillaries of the tail of the living tadpole. These vessels were found to respond to mechanical, chemical and electrical stimulation. Of these, chemical stimulation was apparently by far the most effective. Stricker regarded these changes as due to a turgescence of the capillary endothelium such that, without change of the outside diameter of the vessel, the lumen was altered in size due to a thickening of the wall (7).

Although Cohnheim (8), among others, contested Stricker's view that the capillaries possess inherent contractility in the sense just defined, confirmatory evidence was quickly forthcoming. Stricker's work was followed by that of Golubew (9), who confirmed the observation that capillaries change their size under varying conditions, and advanced the observation that certain spindle elements on the capillary walls contract into spheres, thus occluding the lumina of the vessels and so functioning as a constriction. This notion of the mechanism of capillary function was supported a few years later by Tarchanoff (10). This author used alcohol, ether, ammonia, ferric chloride and acetic acid as well as heat. These procedures caused the so-called spindle elements to swell and so to occlude the lumina, but it was not uncommon to find that the vessels failed to dilate after the stimulus was removed.

In 1878 Severini (11) published a monographic study of the capillaries in which he states, among other things, that the application of oxygen causes the capillaries to contract, while the application of CO_2 causes them to dilate. Severini appears to be of the opinion that these gases act chiefly on the spindle elements described by Golubew. The observations were made upon the frog but also upon the capillaries in the mesentery of the guinea pig. Tarchanoff was unable to confirm these observations of Severini's, nor was Roy, working with von Mehring in 1879, able to confirm them. In 1879 Roy and Brown (12) published their very important paper describing observations on the capillary blood pressure in the frog. These authors were convinced that the capillaries have the power of independent contractility and that their caliber is not, as was believed by many, due to passive changes. They found that there was little difference in the size of the capillaries of the frog's web before and after the leg was amputated. Dilatation of the capillaries was also observed on the application of chloroform, and it was also noted that the capillaries dilated as the result of Goltz's "klopfversuch." These authors were of the opinion

that it is the whole capillary and not the spindle elements of Golubew which contract. They observed that local anemia produced by compression of the area under observation results in a subsequent hyperemia which is accompanied by a dilatation of arterioles, capillaries and venules. This latter phenomenon occurred after section of the sciatic nerve when stimulation of the nerve caused a strong contraction of the arterioles. It is interesting that they observed only a contraction of arterioles on nerve stimulation. Since this procedure failed to elicit changes in the capillaries, they conclude that the functional activity noted in the latter vessels must be due to some local mechanism and they favor the conception that it is due to a direct chemical action upon the endothelium rather than upon the functional existence of what they refer to as peripheral vasomotor ganglia. The latter conception accords with what we now speak of as axon reflexes, which Krogh has recently invoked to explain localized dilatation of capillaries following punctate stimulation (13). More recently Biedl (1894) saw the peripheral vessels (arterioles, capillaries and venules) all contract in the frog's mesentery on the application of salt solution at 45°C., and dilate again when the heat was removed (14).

Mayer in 1885 (15) again observed the endothelium of the capillaries to contract under electrical stimulation. The evidence that the lymphatic vessels throughout the body are contractile is very strong. The phenomenon was observed by Mayer and by Elliott Clark (16) and others in the tail of batrachian larvae. Histological evidence makes it perfectly clear that the lymphatic capillaries are supplied with nerve fibers. These fibers have been observed and pictured a number of times (Kytmanof, 17), and Camus and Gley (18) have obtained graphic records of the functional response of the larger lymphatics to electrical stimulation of nerves. Sabin, in a comprehensive article on the origin and development of the lymphatic system (19), has collected these data in a convincing manner. The lymphatic and blood capillaries are essentially similar in histological structure. The evidence that the blood capillaries are innervated is not so well established as is the case with the lymphatics, nevertheless there appears to be little doubt of the fact. Shäffer states that nerve fibers may be found to follow each individual blood capillary in the rabbit's mesentery (20). Anatomical knowledge therefore furnishes adequate grounds for the expectation, supported by experimental work on the frog, that the mammalian blood capillaries will be shown to be contractile and under the control of the nervous system.

In 1903 Steinach and Kahn (21) published an extensive paper on the contractility of the capillaries. Their observations were directed almost wholly to the effect of direct electrical stimulation in the frog. They used the excised nictitating membrane from the frog and observed, contrary to the findings of Stricker and Biedl, an actual collapse of the whole capillary tube. According to these writers the collapse is due to activity, not of the endothelium as such, but of the perivascular cells described by Rouget (22) and Mayer (15) so that the endothelial capillary tube is collapsed by a passive infolding of the tissue. The venules were also observed to contract.

Analogous results were demonstrated in the omental capillaries of kittens and guinea pigs. The technical procedure used for these animals is not described and capillaries of less than 10 μ were not seen to contract. They believe that the vessels which they actually saw contract belong, however, to the category of capillaries.

Finally, in a frog preparation which is described in great detail, they obtained contraction in the capillaries of the nictitating membrane upon stimulation of the sympathetic fibers which leave the cord in the third, fourth and fifth spinal roots. The latency of contraction was four to five seconds, sometimes twenty seconds, as compared with a latency of one to three seconds by direct stimulation. It was not uncommon to observe rhythmic contractility of the capillaries after the stimulus had ceased. Galvanic was more efficacious than faradic stimulation and it appears that an exceedingly powerful stimulus was required—twelve Daniell cells.

In recent times attention has been again focussed on the functional significance of the capillary bed largely as the result of the work of Dale and his collaborators in the study of histamine shock (23). Dale has advanced the hypothesis that histamine is an endothelial poison which paralyzes the capillary wall so that a marked dilatation occurs. With this dilatation there results a pooling of blood in the capillaries sufficient to account for the marked fall in blood pressure found in conditions of shock. As the result of the work of Bayliss (24) and Cannon (25) and of Abel and Kubota (26), it would appear that a histamine-like substance may be produced in the body as the result of tissue injury sufficient, under certain conditions, to account for the primary symptoms of shock. So that today the opinion is quite widely held that histamine or a histamine-like body plays an important part in functional processes of the body. This applies not only to pathological states such as shock, but Abel and Kubota have advanced the

conception that such a substance is significant in normal physiological processes regulating the distribution of the blood.

In an important contribution to this subject, Krogh (1) has recently shown that an enormous increase in patent capillaries may be demonstrated in active muscle as compared with resting muscle. This increase may indeed amount to more than 700 per cent. Krogh noted that electrical stimulation and massage open up many new capillaries and that electrical stimulation enlarges the capillaries already patent. He regards the mechanism for the regulation of the capillary capacity as being in the capillaries themselves; that is, as due to some chemical regulation or else as associated with an axon reflex mediated through the sensory nerve fibers. He observed that scratching the tongue of an urethanized frog with a glass needle caused a local hyperemia with dilatation of the capillaries and arterioles (13). A closed capillary thus made to dilate opens from the venous end with the appearance of a reversed corpuscular flow. When the dilatation reaches the arterial end of the capillary, the blood stream suddenly assumes the normal direction. Since cocaine was found to abolish this reaction, Krogh was forced to the assumption that it was an axon reflex. He found that cocaine also abolishes the dilatation caused by the application of iodine but does not affect the dilatation caused by acids. Section of nerves supplying the part under investigation at first did not affect the response to chemical and mechanical stimulation. For some days after the nerve section the tissues were hyperemic and the capillaries responded normally to stimulation. Later on, however, the hyperemia disappeared and the capillaries reacted only with a very localized response. From these and other observations Krogh concludes that the capillaries dilate and contract independently of the general blood pressure, and that the spread of response in the capillaries is due to a local axon reflex, probably along the sensory fibers.

Another line of evidence for the contractility of the capillary is found in the tache originally described by Marey (27) in the human being. If a blunt-pointed instrument is drawn across the skin a white line is left which turns to red in a few seconds. In certain cases the red line may be bordered with white and develop a definite urticarial wheal. Clinically this condition is regarded as indicative of vasomotor insufficiency. Marey explained it as due to a localized contraction and dilatation of the capillaries. Bloch (28) thought there was no evidence that the capillaries actually contracted. According to him the red represented capillary dilatation while the neighboring pallor was

due to a drainage of blood into the vessels which dilated because of the mechanical injury.

Ryan (29) has sought to standardize this test for application in various forms of fatigue. Cotton, Slade and Lewis (30) found the reaction well developed in cases of soldiers with irritable heart and adduced new evidence in support of the belief that it is a capillary phenomenon. The red line and neighboring pallor may still be demonstrated after the circulation in the arm has been shut off with a sphygmomanometer cuff. Hence they conclude that the arterioles and venules cannot participate in the production of the red line because there is no excess pressure available to dilate these vessels. If the arm be raised before the pressure cuff is applied the reaction cannot be demonstrated because the skin is depleted of blood. On the other hand, if the arm be lowered before the pressure is applied, the reaction is vivid because there is an excess of blood in the skin.

These investigators incidentally show that epinephrin constricts the capillaries. When epinephrin is injected into the skin the resulting pallor is to be regarded as due to a contraction of the arterioles which shuts off the blood from the distal capillaries. If such an injection be made, however, after the blood flow in the arm has ceased as the result of occlusion by a blood pressure cuff, pallor is still produced. Since constriction of the arterioles would now have no effect in stopping the flow of blood into the capillaries, the pallor which results must be due to constriction of the capillaries themselves. This observation that the local application of epinephrin constricts the capillaries is especially interesting in conjunction with the work of Sollmann (31) who showed that histamine also locally applied develops an urticarial wheal.

Very recently Thaysen (32) has reported quite remarkable oscillations in the red cell count in a case of polycythemia. On one occasion he recorded an increase from 5.3 million to 10.6 million in the course of twelve hours. His investigations and tests showed that these fluctuations were caused by varying contraction and dilatation of the capillaries and precapillaries of the skin. This writer is reported to believe that careful observations would reveal similar results in other cases of polycythemia in which the vasomotor system is so unstable that the condition might be called one of vasomotor or capillary ataxia. These observations are an extreme instance of the well recognized difficulty in obtaining consistent red cell counts in many clinical cases. It would be of very great interest and importance to know if such fluctuations are indeed due to alterations in capillary tone.

METHOD

The technique followed in the experiments which form the basis of this paper was very simple. Cats were used exclusively. After anesthesia was established the animal was placed on a flat holder so that the back of the head and ears rested on the same plane with the vertebral column. The ear to be observed was shaved and thoroughly cleaned and dried. By means of a heavy thread through the upper lip the head was so held that an ear flattened out against the board. This flattening of the surface was further accentuated by sealing the ear to the board with collodion. A microscope giving a magnification of about 70 × was adapted for adjustment over the animal holder and a strong artificial light arranged to give direct illumination of the area at an angle of approximately 45 degrees completed the equipment. Castor oil was flooded over the field at the outset and occasionally during the observations.

With such an arrangement a flat vascular plexus can easily be found preferably in the neighborhood of the tip of the ear in which the corpuscular flow in the finer vessels can be readily followed for an indefinite period. In this region the skin lies loosely attached to the connective tissue which forms the framework of the ear. There is no muscle other than that in the blood vessels which might indirectly influence the vascular area under observation. Animals with little pigment in the skin are preferable but it is far from impossible to obtain good visualization of the capillary network and venules even when a considerable amount of pigment is present. One sees vessels of various size, capillaries with red cells streaming through in single file up to larger vessels in which the corpuscles are packed in a thick column. The picture is essentially like that seen in the transparent tissues of the frog. Vessels exhibiting pulsation are infrequent; probably the arteries and arterioles take a deeper course while the capillaries, venules and veins lie quite superficially, as is indicated to the unaided eye. The capillaries ramify freely particularly about the hair follicles and considerable areas are readily found in which the network forms a horizontal plane and therefore is easily visualized without change of focus. It should be emphasized that with the magnification employed one does not see the capillary wall; it is only by the presence of the red blood cells that the capillaries are recognized. Consequently when the vessels are emptied of blood the field becomes a blank so far as the smaller vessels are concerned.

Attention may be directed to the fact that our present conception that the red blood cells course through the capillaries in single file rests upon observations of the capillary circulation in the frog. In this animal the corpuscles are extremely large as compared with the same cells in the mammal (frog 22 x 16 μ, cat 6 μ, man 8 μ) so that the reason for the prevalent opinion is obvious. In the mammalian capillary circulation, including that of man (33), corpuscles may sometimes be seen moving in this manner but it is much more usual to find the capillaries with lumina sufficient to allow more than one corpuscle to pass at a time. This difference is doubtless due to the higher rate of metabolism in the mammal. There is likewise a much more rapid movement of the corpuscles in the warm than in the cold blooded animals. Therefore to restrict the capillary, in the mammal, to blood vessels in which the corpuscles move slowly and in single file is not strictly consonant with the facts.

Post-mortem behavior of vessels. If in the preparation as above described ether be poured down the tracheotomy tube and the animal be thus killed, the movement of blood at first comes to a sudden stop. Then the corpuscles clump slightly and shortly begin to move forward in the normal direction. This movement, at first noticeable in the capillaries, extends to the venules and there is a slow and gradual progression of blood toward the larger veins. The appearance is as if no blood entered the capillaries from the arterial side and that a milking process, akin to peristalsis, swept the corpuscles onward toward the vein. When the process is complete it may be found that here and there in the capillary net a few clumped corpuscles are locked as if the constriction of the vessel had failed to carry on the last of its contents.

These events occupy varying lengths of time in different animals. They may develop completely in a few minutes or they may last half an hour or more. The completeness with which the vessels empty is also a varying factor. Sometimes, particularly in old and debilitated animals, the blood may not be moved at all; sometimes the field is swept absolutely free of blood but more often a few clumps of corpuscles are left stranded, particularly in the venules. The stagnation of these clumped corpuscles is significant in that they give direct evidence of the contraction of the venules since their diameter is readily appreciated to be less than was that of the same section of the vessel prior to death.

The condition of the vessels as thus described prevails for some time, fifteen minutes or more. Then a remarkable change occurs for the vessels begin to relax and fill. Close observation reveals that relaxation first develops on the venous side and that the blood flows, slowly at first, then quite rapidly, from vein to venule to capillary, and that by the end of approximately an hour the vascular area is filled again and filled full with the indication that capillaries invisible before death are now widely open.

One further change remains to be mentioned: an indefinite time after the relaxation and filling of the capillaries just described, the vascular net is once more emptied of blood. This change is roughly coincident with the onset of skeletal muscle rigor and after it is once developed there is apparently no tendency to a reversal, at least after four days there was in one instance no sign of blood in the smaller vessels.

The foregoing description of the post-mortem appearances of the peripheral vascular bed is substantiated by the photograph reproduced in figure 1. In the technique of photographing these vessels two major difficulties had to be overcome, especially when applied to the living animal. The first was to provide sufficient illumination without heat to cut down the exposure time so as to avoid accidental movements. This was accomplished by the use of an arc light projection lantern without the bellows and projecting lenses. The lantern was tilted at approximately forty-five degrees and so placed that the light rays were concentrated almost to a focus on the part to be photographed at a distance of about 60 cm. from the source of light. The second difficulty was to overcome the movements of the ear due to respiration which, however slight, were sufficient when magnified to spoil the picture in the requisite exposure of thirty seconds. In some animals the respiration was quiet enough not to be a disturbing factor but in the majority of cases a clear picture could not be obtained. In the latter, resort was therefore had to artificial respiration for a couple of minutes before the picture was taken. The apnoea which resulted was adequate to the requirements.

For observation alone the nictitating membrane has some advantages over the ear. In the cat it usually offers a perfectly white ground on which the vessels stand out with exquisite definition. Arterioles, capillaries and venules are readily recognized and less illumination is required. Probably the vessels have less covering tissue over them. Salt solution instead of oil must of course be used to keep the tissue moist. A thread passed through the cartilaginous edge may serve to spread the membrane over the eye ball.

This area of tissue is, however, ill-adapted for photographs. It suffers more than the ear from respiratory movements and the arterial pulsation both in the vessels of the tissue itself and in the underlying eye ball cannot be overcome. In addition to these difficulties activity in the smooth muscle of the membrane itself cannot be controlled with the result that the focal plane cannot be maintained. Finally, and most important for the work in hand this tissue cannot be easily brought below the heart level. It follows that passive drainage of the vessels may therefore result if the circulation stops.

The area photographed was magnified ninety times in most of the pictures taken. A larger magnification than this led to trouble because slight differences of position of the plate, which could not be avoided, resulted in a poor focus. The vessels were focussed on the grownd glass plate of the camera at the beginning of an experiment and as a rule this focus was not changed. A focal error frequently crept in, however, due it is presumed to changes in turgidity of the underlying tissues so that the results so far as the relative size of a vessel is concerned are not wholly trustworthy in the case of capillaries. Conspicuous changes in the size of the larger vessels may be relied upon because such changes can be readily recognized by the eye when using the microscope without the camera. As to the capillaries, their presence or absence in the picture should be the sole criterion. If a capillary has disappeared or if its continuity is broken it is proper to assume that it has constricted because, as has been stated, the vessels under inspection lay below the heart level and only active constriction could empty them of corpuscular elements.

Using the procedure above outlined, photographic records were obtained from eight cats in which no preliminary steps were taken other than etherization and tracheotomy. The animals were kept under ether until a satisfactory control picture was taken and then sufficient ether was poured down the trachea to cause prompt death. Of these eight animals three failed to show a primary vascular constriction shortly after death and were not observed further, and six showed complete or partially emptied vessels. The latter group all showed a subsequent peripheral vascular dilatation followed later by emptying. In addition a number of other animals were observed, but not photographed, with confirmatory results.

Figure 1 is selected to exemplify these results. If shows the four stages of vascular change. Three minutes after death the venules are constricted and the capillaries largely emptied. Forty-five minutes

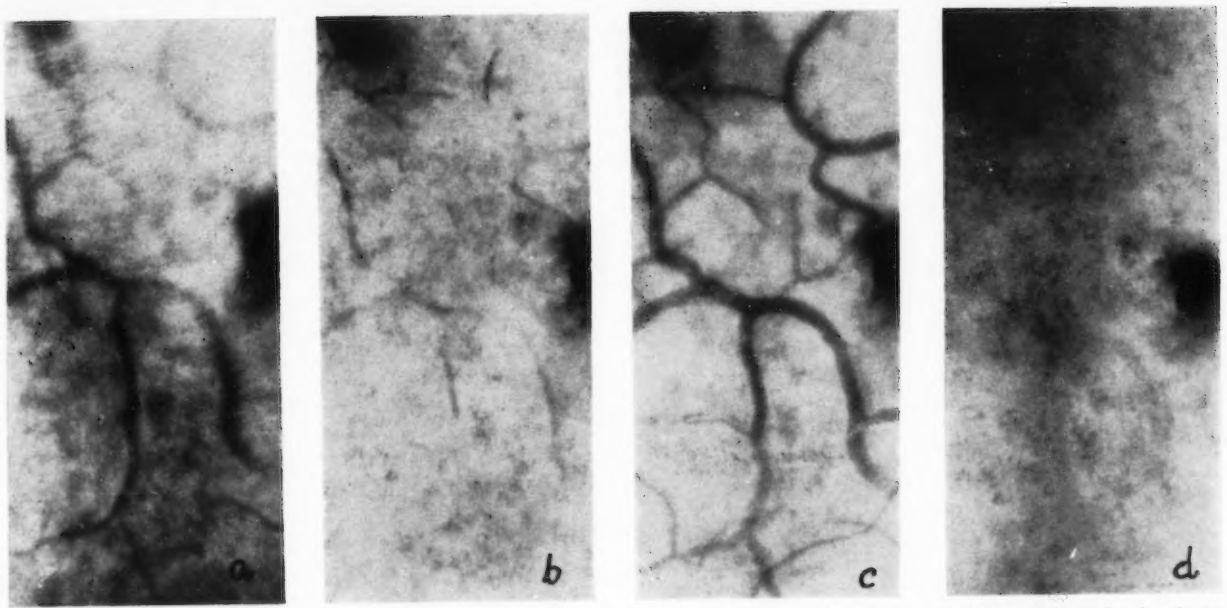

Fig. 1. Post-mortem changes in the peripheral vessels. Magnification 90 ×. Cat. *a*, At 10:28. At 10:36, ether to death. *b*, At 10:39. *c*, At 11:23. *d*, At 12:00. No further change was noted in four subsequent days during which the preparation was preserved. The negatives have not been retouched.

after death the venules are widely dilated and many capillaries are visible. After another period of forty minutes all the blood has been swept out of the vessels and only the shadows of the largest vessels can be found. The data at hand indicate that this last change is permanent since no further alteration was observed to occur for a period of four days.

It may be pointed out here, as was emphasized by Bayliss (20), that there is no inherent difficulty in the conception that the protoplasm constituting the capillary endothelium undergoes change of shape and so mediates a constriction of the vascular lumen. The motility of amoebae and of the white blood cells of higher animals is dependent upon such a change of form and many other similar instances could be mentioned.

That the vascular phenomena above described are not dependent upon the innervation of the vessels was shown in three animals in which death by ether was produced subsequent to section of the cervical sympathetic. In each of these cats the primary constriction was well developed in from one to four minutes. This was followed by dilatation and subsequent constriction in two of the three. In the one which failed to show dilatation the vessels were largely empty three and a half hours after death.

Death by ether subsequent to the intravenous injection of a dose of ergamine phosphate sufficient to cause permanent or transient "shock" (23) is not followed by the same vascular changes. The intact animal or the animal after section of the cervical sympathetic exhibits a primary constriction in the capillaries and venules followed by dilatation and subsequent permanent constriction, as above indicated. If the dose of ergamine is a fatal one or if the animal be killed with ether after partial or complete recovery from "shock," the primary constriction is inconspicuous or wholly absent. In four animals thus observed, two showed no primary constriction whatever and two gave a mere suggestion of it. Furthermore in but one of these animals (a kitten killed by the ergamine) did the constriction subsequent to dilatation develop. In all but this last animal the vessels remained dilated and filled with blood as long as observed (in one case nineteen hours).

These results then clearly support the view advanced by Dale that ergamine phosphate (histamine) is a capillary poison. Additional evidence is found in a single animal in which after histamine and nerve section the vessels similarly failed of the usual response after ether death. In other words, the histamine effect is not dependent upon the integrity of the vascular nerves.

The results thus far presented show that the peripheral vascular bed passes through a number of active changes subsequent to death and that these changes depend upon a local or peripheral function since they are not affected by nerve section and are largely done away with by the injection of an endothelial poison (histamine). Almost at once after death the capillaries, venules and, presumably, the arterioles constrict with the result that the peripheral field is swept more or less completely free of blood. Since the corpuscles can be seen in transit toward the veins, the result is suggestive of peristaltic constrictions running along the vascular tubes. This condition must be looked upon as the first consequence of asphyxia and may well be a significant factor in the asphyxial rise of arterial and venous blood pressure as ordinarily recorded whereby the lesser and minute vessels throughout the body discharge their contents into the larger channels. This passing constriction gives way shortly to a marked dilatation such as is usually associated with the collection of asphyxial and catabolic products. Some time later and roughly coincident with the onset of skeletal muscle rigor a second constriction develops which is apparently permanent in character. Whether this change is due to a rigor contraction of smooth muscle and endothelium, our present knowledge of these tissues is insufficient to determine. In no case were observations continued long enough (never more than four days) for skeletal muscle rigor to pass off, consequently the assertion that the change, assuming it due to rigor, is permanent is somewhat arbitrary. Post-mortem tissue changes, including laking of the blood, may in this length of time, however, have so clouded the field that the vessels could not be seen even though they were filled with blood. Also the blood may clot and so fail to run into the vessels even after their lumina have become patent. It will be noted that this observation does not accord with the livor mortis seen so frequently by pathologists.

Experiments with nerve stimulation. A second point of even greater interest and importance brought out in this research is the effect of nerve stimulation upon the peripheral vascular bed. In this set of observations the animals were usually anesthetized with urethane and the cervical sympathetic nerve dissected out for stimulation. In no instance was it possible to determine, by inspection or by photographs, that section of this nerve altered in any way the caliber of the vessels of the ear. On the other hand, electrical stimulation of the nerve gave unmistakable evidence of constriction in both capillaries and venules and subsequent to stimulation an over-dilatation was

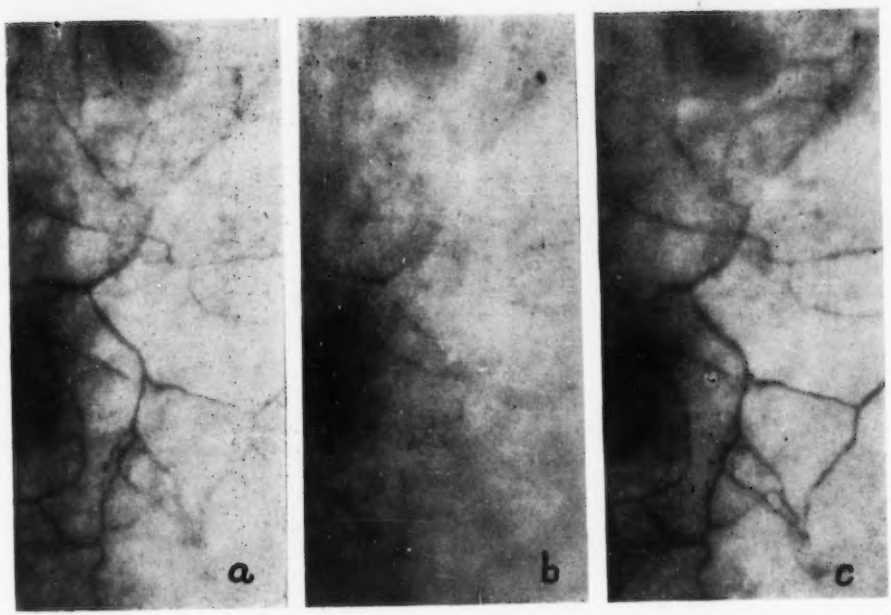

Fig. 2. Constriction of capillaries and venules by electrical stimulation of the cervical sympathetic. Magnification 90 ×. Cat. *a*, Before, *b*, during, and *c*, after stimulation. The negatives have not been retouched.

recognized. These results were so sharp that it was considered necessary to perform but three experiments in each of which the observation was repeated many times. No indication of fatigue was noted since the results were as good five hours after an experiment was begun as at the start. Figure 2 gives the photographs obtained in one of these experiments.

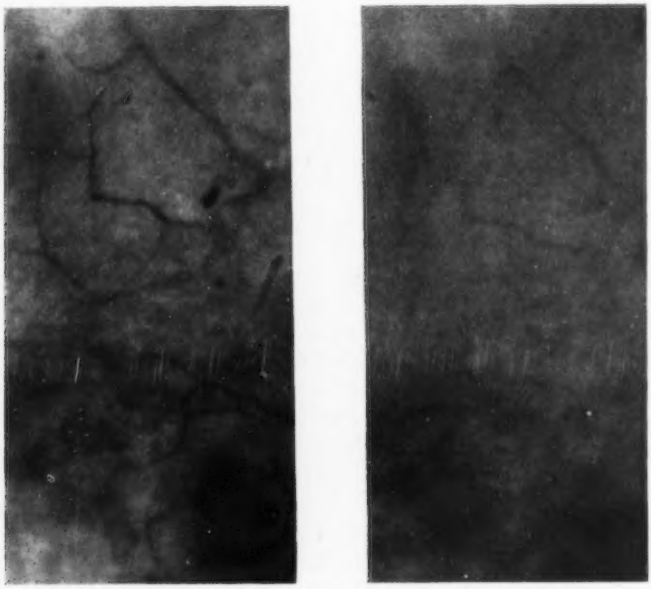

Fig. 3. Constriction of capillaries and venules following the injection of 3 cc. 1: 50,000 epinephrin. Magnification 90 ×. Cat. The cervical sympathetic nerve had been cut. *a*, Before, and *b*, shortly after the injection of epinephrin. The negatives have not been retouched.

This figure shows clearly the constriction and disappearance of the capillaries and venules during electrical stimulation of the cervical sympathetic and their subsequent over-dilatation two minutes after the stimulus was removed. It happens that no larger venule was present in this field but these were repeatedly seen to respond just as conspicuously as shown here for the capillaries. The response of the venules may be appreciated, although not very clearly, in the next figure (fig. 3), which shows the effect of epinephrin injection.

Experiments with epinephrin. Further evidence of the sympathetic innervation of the capillaries and venules was developed from the injection of epinephrin. This substance, selective in action for contractile tissues with sympathetic nerve supply, gave results comparable with those obtained with electrical stimulation of the cervical sympathetic. The effect was the same both before and after section of the sympathetic. Figure 3 is made from photographs taken before and just after the intravenous injection of 3 cc. of 1:50,000 epinephrin in a cat three and a half hours after the cervical sympathetic had been cut.

The injection of histamine destroys this mechanism. In a cat in which nerve stimulation had given sharp constriction, 6 mgm. ergamine phosphate were injected. As soon as the resultant dilatation of the peripheral vessels was developed, the cervical sympathetic was again stimulated. The strongest stimulating current available failed to elicit the slightest response.

The findings here presented are contrary to our present belief that the active functional peripheral resistance is to be found wholly in the smaller arterioles with smooth muscle in their coats. The evidence given indicates that nerve impulses along vasomotor fibers may play upon the caliber not only of the arterioles but on that of the capillaries and venules as well. We must therefore modify our conception of the peripheral resistance in the matter of functional activity to include the whole peripheral vascular bed including therewith the arterioles, capillaries and venules.

It will be obvious, furthermore, that if the conception in regard to the peripheral resistance here advanced is substantiated, the body possesses a remarkable mechanism for the regulation of the distribution of the blood. For by alterations in the tonic capacity of the capillaries and venules, which is under the control of the central nervous system, circulating corpuscles as well as plasma can be mobilized to a very considerable degree in accordance with the physiological needs of the various tissues both local and general. It would seem also to follow that blood volume and plasma volume determinations must be subject to the same physical mechanism (34).

If, as is highly probable, specific nerve fibers supply the different parts of the peripheral vascular bed (arterioles, capillaries and venules) the play of functional adjustments must be exceedingly complex. Hitherto it has been possible to invoke chemical processes alone to account for many of the exquisite adaptations recognized to occur in

physiological adjustments. In the light of the facts here presented we may conceive of a highly organized nervous mechanism adapted to quick and efficient response superimposed upon the primitive chemical methods available to the organism. There is doubtless a happy coaptation between these two major processes of control but on the body surface, exposed to noxious environmental factors, and in the voluntary muscles where quick adjustments of blood supply are constantly demanded, we might expect the nervous regulation to play a significant teleological rôle. In the glands and deeper body tissues generally, on the other hand, where reaction time is of less significance, responses may largely depend upon chemical factors for their instigation.

If the body has at its control such a highly organized device for the disposition and partition of the blood by which extensive capillary beds may be largely emptied of or packed with corpuscular elements or plasma, an explanation is readily found for the uncertainties of blood cell counts and blood volume determinations. Individual capillaries may be opened up or closed or they may be gorged with stagnant inactive corpuscles, as is possible to demonstrate on the finger (32). Mediation of these and similar changes would be accomplished by activation of the arteriole, capillary or venule functioning individually or collectively. It is natural to infer that normally such forces are well balanced and counteract one another so that the volume and corpuscular composition of the blood is held relatively stable, but in time of physiological stress or in disease the alterations which develop might assume considerable proportions. The significance of this mechanism for the nutrition of the tissues will likewise be apparent since it may be presumed to exercise control over the rate at which plasma passes through the capillaries and into the tissue spaces.

The present work does not include a demonstration of nervous regulation of vasodilatation in the capillaries and venules but the evidence justifies the assumption of such an hypothesis. On the other hand, the primary constriction followed by dilatation which occurs after death indicates that chemical regulation may function both in constriction and dilatation. The effects of the injection of epinephrin and of histamine likewise substantiate this conception. These findings accord with the recent clean-cut results obtained by Krogh (1) on the increase in number of patent capillaries in active muscle and the similar results recognized to occur in the early stages of inflammatory processes (4). The work of Gaskell (35), Bayliss (36) and the writer (37) on the chemical regulation of peripheral resistance is thus to be interpreted

that chemical factors constrict as well as dilate the finer vessels other than the arterioles.

To further substantiate the fact that nervous impulses actually produce a constriction of capillaries and venules, attention may again be called to the condition of these experiments. The vascular bed under observation lay some 2 cm. below heart level. An occlusive constriction in the arterioles could not therefore passively drain the vessels distal to the constriction and supporting a hydrostatic column. It is conceivable that, if these vessels were under tension due to a *vis a tergo*, they might decrease in size when their filling pressure was shut off but they would not empty. Indeed it was found in an experiment in which the carotid was occluded long enough to bring the blood stream to a standstill in the peripheral vessels, that the capillaries showed no appreciable decrease in size and that the corpuscles did not tend to clump. The latter point is small but significant because when the capillaries are made to contract, as by nerve stimulation, the corpuscles invariably tend to gather together and move along in clumps.

Lister (4) in that splendid paper to which reference has already been made on the "Early Stages of Inflammation" published in 1858, describes an experiment on the frog which is of decided interest in this connection. He was studying with the microscope the behavior of the peripheral vessels in the web of the foot under various conditions and in this experiment he observed that irritation of the cord caused the capillaries and venules to disappear from view. He ascribes this result to an active constriction of the aterioles sufficient to block the passage of the red cells but insufficient to stop the flow of plasma so that the corpuscles floating in the capillaries and venules are washed onward from the field. The red blood cells of the frog are of course relatively large and could presumably be blocked in the manner indicated, but I am inclined to believe that Lister actually saw a constriction of the vessels in question. In the first series of experiments described in the present paper the evidence is clear that, after the heart has ceased to beat, the capillaries and venules can empty themselves not once but twice against an appreciable hydrostatic resistance. The movement and disposition of the corpuscles under these conditions is not to be distinguished from their behavior under the influence of nerve stimulation; the stream stops quite abruptly, the corpuscles congregate in masses and then progress slowly and without definite regularity. This forward movement occurs against a hydrostatic resistance and in spite of the fact that the corpuscles would tend in a stagnant plasma, because of their specific gravity, to settle and adhere to the vessel wall.

Doctor Connet has recently shown in a research done in this laboratory (38) that the injection of epinephrin raises the systemic venous blood pressure. In this work she was able to exclude the slowing of the heart which has hitherto been regarded as sufficient to account for the phenomenon, and reached the conclusion that the substance acts by a direct effect upon the veins in the intact animal just as it has been repeatedly shown to act upon isolated vein preparations. It seems highly probable in view of the results presented here that the rise in venous pressure following the injection of epinephrin demonstrated by Doctor Connet is associated with a constriction of the capillaries and venules as well as of the veins.

Experiments with histamine. With a method available for the study of the capillaries in the mammal it was quite natural that one should be led to a study of the effect of histamine. The attractive hypothesis advanced by Dale (23) that histamine "shock" (and probably traumatic "shock") is due to a specific toxic action upon the capillaries rested upon indirect evidence. It was possible with the preparation at hand to put this hypothesis to a direct test.

References have already been made to the toxic action of histamine on the capillaries and venules in the experiments previously described:—after the injection of histamine post-mortem constriction of these vessels was absent or very slight and no constriction could be obtained by nerve stimulation. In addition to these experiments a number of experiments were performed, seven in all, in which attention was directed primarily to the histamine effect. Ergamine phosphate, 6 mgm. per kilo in salt solution, was injected intravenously in accordance with Dale's technique.

The results were uniformly clean-cut and decisive. Within a few minutes after the injection the capillaries and venules were filled with stagnant blood and definitely dilated. The dilatation was distinctly more conspicuous in the venules. These changes developed in conjunction with the fall in arterial blood pressure and in one experiment in which it was followed with a fall in venous pressure.

Doctor Rich has obtained similar results in the capillaries of the mammalian omentum (39) by a different method. Rich found that flooding the peritoneal cavity with Zenker's fluid gave prompt fixation of the tissue so that it could be removed and studied under the microscope. If the tissue was thus fixed immediately after the intravenous infusion of histamine, marked capillary and venous dilatation and engorgement could be demonstrated. This vascular change was entirely absent in

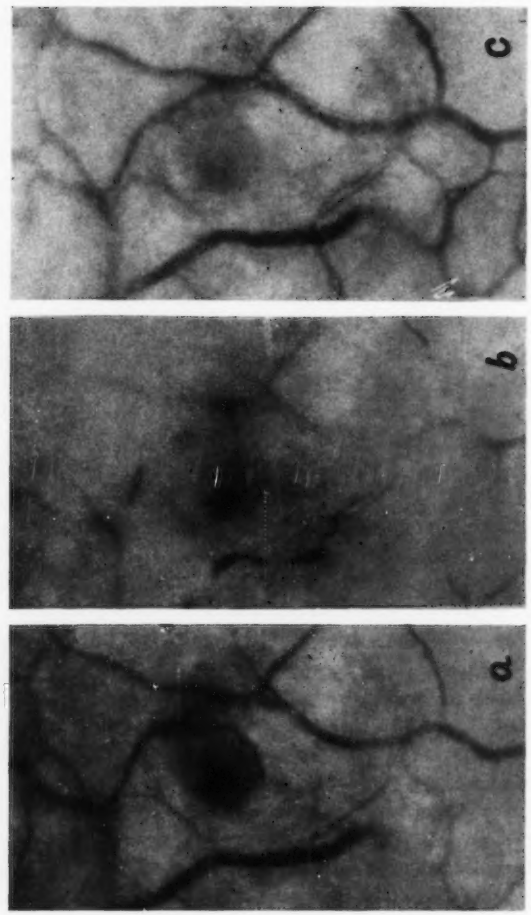

Fig. 4. Effect of histamine on the capillaries and venules. Magnification 90 ×. Cat. *a*, At 10:28. At 10:40, ergamine phosphate (6 mgm. per kilo) was injected. *b*, At 10:50. *c*, At 11:25. The negatives have not been retouched.

control experiments in which salt solution was infused instead of histamine.

Usually, although not invariably in my experiments, this dilatation was preceded by what must be interpreted to be a constriction, under the experimental conditions. This constriction lasted a variable but brief period of time, frequently so short that it could not be photographed satisfactorily. This reaction is especially well exemplified in figure 4 in which the photograph which shows the constriction was taken ten minutes after the injection of ergamine when the arterial pressure was 24 mm. Hg. The arterial pressure was still at 24 mm. Hg. thirty-five minutes later when the last photograph shown in this figure was obtained.

This transitory constriction of the capillaries and venules may not occur throughout the body since Rich was unable to find any evidence of it in the capillaries of the omentum. It may be due to a primary central effect of the poison or, what seems more probable at the moment, it may represent one of those curious reactions according to which a drug or substance depressive in effect at first acts as a stimulus. Such a transitory reversal of effect is not uncommon in perfusing the isolated heart with inorganic salts and I have observed a similar effect in perfusing the respiratory center (40). Burridge (41) has suggested, in the case of the heart, that the condition is associated with the state of aggregation of the colloids.

Although it thus appears possible that histamine may under certain conditions produce its primary "shock" effect while the capillaries and venules of the ear are constricted, these experiments as a whole undoubtedly lend strong support to Dale's hypothesis to explain histamine "shock."

In conclusion it will not be out of place to state that the preparation used in these experiments offers an excellent method of demonstrating the capillary circulation in the mammal to students. A low power microscope (ocular 1 and objective 3), adapted by removal of the stage to fit over the edge of an animal holder and a good light are all the apparatus that is required. The capillary circulation can be similarly observed in the rabbit and presumably also in the dog although the latter animal has not been investigated. The rabbit's ear is, however, distinctly more susceptible to inflammatory processes than is that of the cat.

SUMMARY AND CONCLUSIONS

A method is described whereby the peripheral circulation (particularly the capillaries and venules) in the cat's ear may be observed and photographed. It is thus possible to study in the living mammal the capillary circulation, investigation of which in the intact animal has hitherto been limited to the frog. Making use of this method, the following experimental results were obtained:

1. After ether death the peripheral vessels at first constrict so that they are largely emptied of blood. This constriction which occurs usually within a few minutes lasts but a short time and is followed by a marked dilatation and engorgement. Subsequently and roughly coincident with the development of skeletal muscle rigor, the vessels are again emptied of blood. The latter condition prevails indefinitely (four days at room temperature).

These changes are much less conspicuous or entirely absent if the animal is previously injected with histamine. They are not affected by section of the vasomotor nerve fibers (cervical sympathetic).

It is thus concluded that these post-mortem vascular reactions are independent of the central nervous system and that they are abolished by an endothelial poison.

It is suggested that the first constriction represents a response to asphyxia and may be a significant factor in the asphyxial rise of arterial and venous blood pressure.

2. Section of the vasomotor fibers to the part (cervical sympathetic) did not cause an appreciable dilatation of the vessels but electrical stimulation of these fibers gave clear evidence, by causing constriction, that the capillaries and venules are under sympathetic nervous control. This fact was further substantiated by the injection of epinephrin which caused a similar vascular response.

The reaction to nerve stimulation could not be obtained in an animal poisoned with histamine.

These results indicate that the functional peripheral resistance is not limited to the arterioles but includes the capillaries and venules as well. It is inferred that this resistance is subject to chemical as well as nervous control. This concept involves a reorganization of our present beliefs concerning the peripheral resistance and implies the existence of an efficient physical mechanism for the distribution and regulation of the circulating blood volume and of the supply of nutriment to the tissues.

3. The injection of histamine (ergamine phosphate) causes a prompt and permanent dilatation of both capillaries and venules with stagnation of the corpuscular stream.

This reaction appears to be characteristic and thus confirms Dale's hypothesis that histamine "shock" (and presumably traumatic "shock") is largely dependent upon the reaction of the capillaries. It should be noted, however, that the present results extend this reaction to include the venules as well as the capillaries.

Prior to the dilatation which appears to be the characteristic histamine effect, there is usually a short period when the vessels in the ear are constricted. Neither this transitory effect nor the general histamine effect is influenced by nerve section. Both effects are thus due to the direct action of the substance on the vessels.

It is probable that vessels elsewhere in the body do not show this passing constriction since it may persist for some minutes after the systemic arterial pressure is at shock level and give place to dilatation while the blood pressure level remains unchanged.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

I. Lung Automatism and Lung Reflexes in the Frog (R. pipiens and R. catesbiana)

A. J. CARLSON and A. B. LUCKHARDT

From the Hull Physiological Laboratory, University of Chicago

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This report is the beginning of an investigation and analysis of the reflexes evoked by the visceral sensory nerves in all the groups of vertebrates available for study. The inception to this line of work was the observation on man (7) that strong contractions of the empty stomach produce reflex effects on the cardiac and the vasomotor centers. To date we have studied the reflexes from the visceral afferent system involving the skeletal musculature,[1] the respiratory mechanism, the gastro-intestinal tract, the heart and blood vessels, and the urinary bladder. In some cases our reflex results compelled us to re-investigate the motor mechanisms of the organ involved in the reflex response. This is true especially of the lungs.

We have today fairly comprehensive and accurate knowledge of the efferent nervous mechanism of the viscera, thanks to the work of Gaskell, Langley and others.

On the sensory or afferent side our information is made up largely of gaps and guesses, despite its probable importance in functional integrations in health and disease. This phase of physiology has been studied especially with reference to *conscious* visceral sensations, to witness only the work of surgeons and internists on direct and referred visceral pain, and of physiologists and psychologists on the sensibility (conscious) of the alimentary canal. To our knowledge a thoroughgoing investigation of the sub-conscious reflexes evoked from the visceral sensory nerves in health and disease has not been made. In Gaskell's recent monograph (8) on the involuntary nervous system the afferent component of this system is not even mentioned, and in Sherrington's article on the "Sympathetic Nervous System" in the 1911 edition of the Enclyclopedia Britannica the afferent component

is dismissed with the following sentence: "Of the afferent fibers of the sympathetic little is known save that they are, relatively to the efferent, few in number, and that they, like the afferents of the cerebro-spinal system, are axones of nerve cells seated in the spinal ganglia."

EXPERIMENTAL METHODS

1. The lung contractions were registered by means of water manometers (diameter 8–10 mm.) connected with small glass cannulae inserted and tied in the tips of the lungs. For the most delicate lung contractions these water manometers were not sufficiently sensitive, and in the study of these phases a very delicate tambour was employed. In fixing the cannula in the tip of the lung care must be taken so that the direct handling of the lung is minimum and gentle, as direct and rough handling induces prolonged tonic contractions that may involve the whole lung. In animals in poor physiological conditions, the lungs are usually quite atonic, and these contractions due to direct handling (mechanical stimulation) are less in evidence.

In experiments with the glottis open and the frog preparation breathing spontaneously no artificial pressure can be maintained in the lungs, because if the lungs are collapsed through cannulae in the lung tips, the frog promptly fills the lung again up to the original pressure. If this original pressure is slightly exceeded by inflation through the cannula the glottis is promptly opened and the pressure reduced. In the experiments involving the closure of the glottis the lungs were practically always collapsed and empty at the conclusion of this operation In the subsequent inflation of the lungs we always took pains not to exceed the normal pressure maintained by the frog (1–3 cm. water).

Most of the previous investigators of the physiology of the respiratory movements in the frog have used various methods for graphic registration of the throat and flank movements (Martin (16), Wedenskii (26), Langendorff (14), Sherrington (23), Baglioni (3), Soprana (24), Nikolides (20), (21)). Brown (5) and Willem (27) recorded intrapulmonic pressure by means of cannulae in the tip of the lungs. Mochi (17), (18), (19) placed the body of the frog, except head and throat, in a plethysmograph and closed the plethysmograph by sectioning the frog's skin around the neck and tying to the plethysmograph tube. It seems to us that the method of Mochi introduces more trauma and abnormal physiological conditions than a slit through the abdominal wall for placing cannula in the tip of the lungs.

2. For the registration of the variations in the intrapulmonic pressure during normal respiration the animals were usually decerebrated, and slits made through the abdominal wall over the lung tips, of sufficient size to insert cannulae in the lung tips. This incision through the abdominal wall was made with or without local application of cocaine. The animals were then placed, usually without restraint, on a board or preferably in a small dark box. In most cases animals thus prepared would sit quietly for long periods, unless disturbed by external stimulations. In a few animals the abdominal incisions were made under local anesthesia without previous decerebration.

3. In the experiments where it was necessary to separate the lungs completely from the influence of skeletal muscle contractions several methods of procedure were used:

a. After decerebration and fixing the cannulae in the lung tips, the animals being placed in normal position (ventral side down) on the board or in the dark box, the abdominal muscles were cut away and the spinal cord pithed below the brachial plexus. In such a preparation movements of the head, strong respiratory movements (swallowing) or movements of the front legs will alter the intrapulmonic pressure, but the rapidity of these movements is much greater usually than the lung contractions so that the latter can be readily differentiated from the passive effects of the former movements, and in favorable preparations the former movements may be absent over considerable periods, thus giving the lung contractions free play.

b. Without previous decerebration the spinal cord was cut just below the medulla and pithed the entire length caudad, the animal placed on the board dorsal side down, the abdominal wall opened for its entire length by a median incision, and the lungs completely isolated, except for their anatomical connections with the pharynx and esophagus. Animals thus prepared continue to breathe spontaneously for considerable periods if care is taken not to injure the lungs or pharynx and prevent exsanguination. Head and pharyngeal movements are still capable of influencing the intrapulmonic pressure mechanically. The isolated lungs were prevented from drying by a thin layer of absorbent cotton kept moist with Ringer's solution.

4. Closure of the glottis. Our greatest technical difficulty consisted in proper closure of the glottis in experiments where this procedure was essential. The frog has no trachea and bronchi. The glottis opens directly into a rather large tracheal sac which communicates with the base of each lung. This tracheal sac is so closely adherent to the tis-

sues at the base of the heart that we found it impracticable to close the lungs by ligation or compression of this sac without an amount of injury to the heart nerves, and main blood vessels that resulted in quick failure of the circulation. The following experiments were tried without practical success:

(1) Ligation of base of lungs at their junction with the tracheal sac, leaving the lung blood vessels and nerves outside the ligature. This failed because of the impossibility of accomplishing the latter without puncturing the lung wall, or if successful the ligation produced enough anatomical distortion to interfere with the lung circulation.

(2) Closing the tracheal sac or either lung by small wads of cotton pushed through the glottis. This failed mainly because the glottis opening is smaller than the diameter of the tracheal sac or its communication with the lungs. Hence the cotton wad passed sooner or later into the lung cavities.

(3) It was noted, when the median incision exposing and isolating the lungs was carried forward to the level of the base of the heart only, that lateral and dorsal tension exerted by pull on the front legs would prevent air from entering or leaving the lungs by the normal breathing movements. This was evidently due to collapse of the tracheal sac by external compression. This gave us the clew to a method of closing the glottis and blocking the air communication between the two lungs with the least possible trauma or physiological violence. A cotton plug of suitable size, with or without a coating of vaseline, was inserted through the mouth and pushed down the esophagus to the level of the glottis and the tracheal sac. The pressure thus exerted on these structures from the esophagus not only closed the glottis but usually compressed the tracheal sac sufficiently to prevent air communication between the two lungs, especially if in addition the front legs were put under slight dorso-lateral tension.

This mode of procedure sufficed for the degrees of lung contractions accompanying the normal respiratory movements, or induced by reflex stimulation. But it proved inadequate in case of the extreme tetanus of the lungs following section of the vago-sympathetic nerves or pithing of the medulla. These strong contractions always forced the glottis open against the cotton plug in the esophagus. Hence in all the experiments on this phase of lung physiology the glottis had to be closed more firmly. This was done by clamping the rim of the glottis with a slender artery forceps. The mouth being held open, a slender hook was passed through the glottis, under gentle forward traction the rim

of the glottis was compressed with a slender artery forceps, a small cotton plug pushed into the esophagus and left in situ together with the forceps. Care must be taken not to place the forceps too far down on the tracheal sac and pharyngeal tissues, as in that case the pulmonary branches of the vagi as well as the lung blood vessels are included in the grip, or placed on such tension that vagus action on the lung and lung circulation are interfered with.

The essential drawback to this procedure is the trauma produced or rather the violent mechanical stimulation of the sensory nerves in the glottis, larynx and pharynx by the compression. Placing the artery forceps in the region described produced something like profound prostration or "shock" in the preparation. Respiratory movements cease for a considerable period, and in the case of animals otherwise in poor condition may not return at all. It is scarcely necessary to add that the results reported, using this method of closure of the glottis, are based on the vigorous preparations in which spontaneous respiration returned.

5. The mucus in the lung cavities can, of course, not be eliminated from the lungs in the normal way under any condition of glottis obstruction. Furthermore, the unavoidable trauma to lungs and tracheal sac in preparation may actually increase the mucous secretion. This lung mucus is a hindrance and a source of error in registering lung tonus and contractions by our method, as strong contractions may force some mucus into the cannula in the lung tips, and this will interfere with the prompt and accurate response of the water manometer to slight variations in the intrapulmonic pressure. It is needless to say that preparations must be discarded in which the lung mucus interferes with accurate recording of lung contractions.

6. Administration of the drugs. All the drugs used, unless otherwise noted, were mixed with varying quantities of Ringer's solution and injected slowly into the abdominal vein. A few injections were made directly into the heart.

7. Prevention of asphyxia after closure of the glottis. It was, of course, essential to maintain circulation and lung ventilation even after closure of the glottis, so that abnormal reflexes and local lung reactions would not be set up by asphyxia. We endeavored to maintain good circulation by ligation of the main blood vessels sectioned in the preparation of the animal and by occasional intravenous injections of small quantities of Ringer's solution. The frog's heart is apparently very sensitive to the mechanical factors of filling, as it ceases to beat entirely or beats

very feebly when the blood pressure is very low, but resumes an adequate rhythm on replacing the lost blood with Ringer's solution.

It is well known that the frog in water carries out a considerable proportion of its gaseous exchange through the skin. Under the temperature conditions prevailing in the laboratory during this work the frogs (R. pipiens) would remain under water for 18 to 25 minute periods, come to the surface and make a few vigorous respirations and submerge again for the same length of time. Evidently the filling the lungs with air, supplemented with the skin respiration, met the respiratory needs for 20 to 30 minutes. On the basis of these facts, we always kept the skin of our frog preparations moist with water, and gave occasional artificial respirations, except in cases of extreme lung tetanus when the latter procedure would have been useless.

8. All the tracings reproduced with this report were taken with the same speed of the kymograph. The time record is not always attached to the tracings reproduced, for reasons of economy of print paper. But the reader interested in any question involving the time element as a matter of importance can readily transfer the time tracing, given in a few of the tracings, to the others; 25 cm. of tracing (original size) = 17 minutes.

LUNG TONUS AND LUNG CONTRACTIONS DURING NORMAL RESPIRATION

1. The anatomy of the frog's lung is well known. The reader will recall that the lung is a paired muscular sac, numerous septa on the interior surface dividing this into small spaces or alveoli. The septa extend only a few millimeters from the lung wall, so that the larger part of the lung cavity is a large single air space. There are no bronchi and no true trachea, the tracheal sac having essentially the same structure as the rest of the lungs, and probably carries out the same respiratory function.

Smooth musculature covers the entire wall of the lungs and extends into the smallest septa on the inner surface. More or less definite external muscle strands follow the course of the main pulmonary blood vessels on the lung surface.

The arrangement of the lung musculature is such that contraction (even of the septal musculature) will reduce the size of the lung cavity, or raise the intrapulmonic pressure in case the air in the lung is not free to escape.

The action of the septal musculature would be analogous to that of the bronchial constrictor muscle of the mammalian lung. So far as we know, the mammalian lung has no counterpart to the lung wall musculature in the frog.

After having discovered the striking peripheral motor automatism of the frog's lung we become especially interested in the local nervous tissue in the lung of this animal group. According to the histological investigations of Arnold (1), Smirnow (25), Cuccate and Wolff (28), there are numerous ganglia, as well as isolated ganglion cells (multipolar and bipolar) along the course of the main vago-sympathetic nerve trunks on the surface of the lungs. There are medullated and non-medullated nerve fibers in these nerve trunks. A plexus of fine non-medullated nerve fibers surrounds the strands of lung musculature. The ganglion cells and these nerve plexuses are most abundant at the base of the lungs. Arnold points out that the ganglia and ganglion cells in the frog's lung are histologically identical with those of the frog's heart. They are also probably identical, both as to histology and function, with the ganglionic plexuses (Auerbach) in the wall of the gut, especially as the lung is a diverticulum from the esophagus.

2. The external respiratory mechanism of the amphibians differs from that of all other air-breathing animals in that the air enters the lungs under positive pressure due to the act of swallowing. One might therefore surmise that in the amphibia the respiratory center in the brain is anatomically and physiologically identical with the center for deglutition.

The sequence and coördination of the respiratory acts (buccal movements, closing of nares, expiration and inspiration or swallowing air) have been correctly analyzed and described especially by Langendorff (12), (13), Baglioni, Brown (5) and others, and most recently by Willem (27). We have nothing new to add on that point, and can contribute no new facts bearing on the old and new speculations as to phylogenetic and physiological significance of the buccal movements which proceed rhythmically between the actual renewal of air in the lungs (swallowing).

The types of the respiratory rhythm as revealed by the intrapulmonic pressure in normal and in decerebrated frogs are shown in figure 1. The filling of the lungs by swallowing (upstroke) is preceded by opening of the glottis and escape of some air into the buccal cavity. According to most of the competent and recent investigators, the nares are closed during the whole act so that while the air escapes from the lung it

does not actually escape through the nares. Rebreathing is therefore a marked feature of the frog's lung respiration. The buccal movements going on between the swallowing acts and with the nares open, bring fresh air into the buccal cavity.

In exceptional cases there is a perfect synchrony between the buccal movements and the actual air swallowing (fig. 1, *B*). But usually

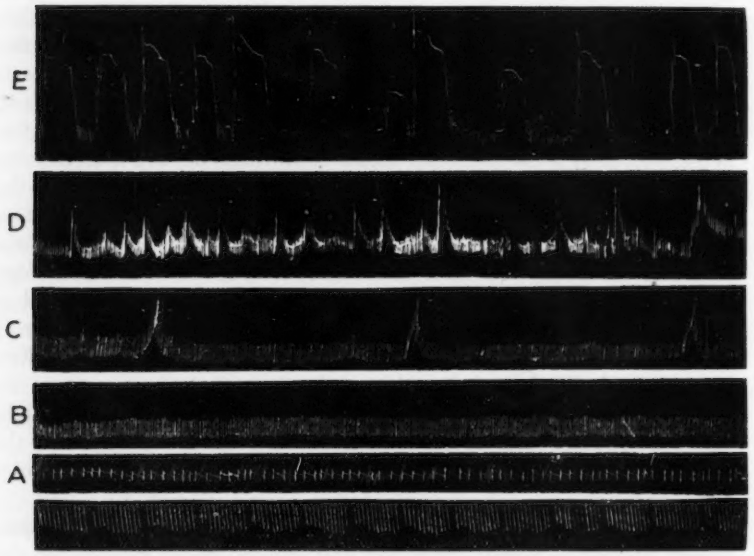

Fig. 1. Records of intrapulmonic pressure in frogs. Tracings *A*, *D*, *E*, taken by water manometer; tracings *B* and *C* by air transmission and tambour. Tracings *A* to *D*, Rana pipiens, animals decerebrated and the tip of one lung exposed by small abdominal incision. No anesthetics, animals sitting quietly in normal posture. Tracing *E* from bull frog (R. catesbiana), tip of lung exposed by small abdominal incision after cocaine, animal sitting quietly in a darkened moist box; time, 5 seconds. Showing varying types of respiration in the frog

All the tracings n this article are reduced to about $\frac{2}{5}$ of the original.

several buccal movements are made between each swallowing act (fig. 1, *A*), and a striking feature of the air swallowing rhythm in most frogs is a periodicity similar to the Cheyne-Stokes breathing in mammals. This has been observed by most of the former investigators on the respiratory movements in the frog (Luschinger and Sakalow (15), Langendorff (12), (13), Wedenskĭ 26, Sherrington (23), etc.). The

frog may go on over long periods swallowing as much air as that which previously escaped through the open glottis, thus maintaining a constant general level of intrapulmonic pressure of 1 to 2 cm. of water. This type may periodically change into one in which during a few powerful air swallowings the amount of air forced in greatly exceeds the quantity that escaped between the time of glottis opening and the swallowing act. In consequence of this the lungs expand and the intrapulmonic pressure rises from the general level of 1 to 3 cm. of water up to a level of 6 to 9 cm. of water. All respiratory movements then cease for periods varying from 5 to 60 seconds and the act is renewed, that is, the quantity of air let out of the lung in each respiratory act is greater than that forced in; the lung shrinks and the intrapulmonic pressure falls to its former general level of 1 to 2 cm. of water (fig. 1, *C, D, E*).

According to our experience, this is the usual type of respiration in the frog. As previously noted by Sherrington and others, decerebration or other methods of preparation are not responsible for inducing it. It is probably a normal rhythm developed in connection with the habitual under-water existence of the animal. During the respiratory pause with high intrapulmonic pressure this pressure, as recorded by the ordinary water manometer, may show an initial rise and then remain at a fairly constant level until the next respiratory act, but if the pause is long the intrapulmonic pressure gradually falls, due, not to escape of air through the glottis, but to relaxation of the tonus of the lung musculature.

3. Active lung contractions and lung inhibitions associated with the respiratory movements.

a. Contractions. The reader's attention is invited to the tracings reproduced in figures 2 and 3. It will be noted, especially on the upper tracing in figure 2, that at the end of the last respiration followed by a Cheyne-Stokes pause, there is a latent period of 1 or 2 seconds followed by a rise in the intrapulmonic pressure that may exceed the maximum upstroke of the final inspiration. When the pause is sufficiently long and registration apparatus sufficiently delicate, it will be seen that this rise in pressure is due to a contraction lasting from 10 to 15 seconds. These contractions are evidently due to the activity of the lung musculature, for we have observed them in animals after isolation of the lungs, fixation or resection of the abdominal and shoulder muscles or destruction of the entire spinal cord below the medulla. Moreover, the changes in the intrapulmonic pressure due to active

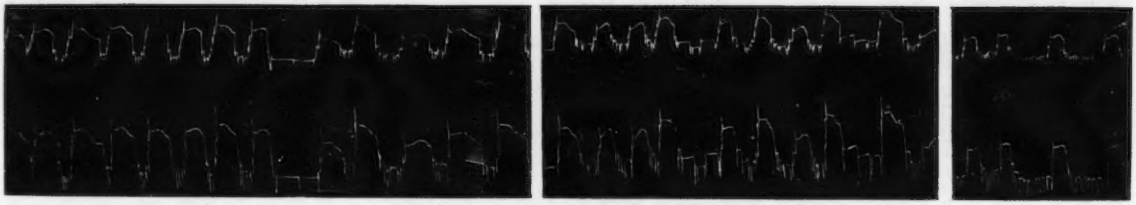

Fig. 2. Tracing of intrapulmonic presure in the bull frog in normal respiration. Frog in normal posture and resting without restraint in dark box. Operation for insertion of cannula in tip of one lung made under local anesthesia. Lower record by water manometer, upper record by delicate tambour. Showing the curve of lung contraction during the respiratory pause following the periodic rapid inspiration. The lung contractions are shown best on the tambour tracings, as this instrument is more delicate than the water manometer.

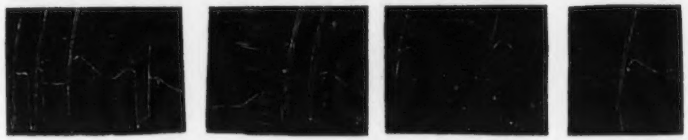

Fig. 3. Rana pipiens. Water manometer tracings showing contractions of lung following spontaneous respiration (quick up and down strokes). Whole brain exposed, spinal cord cut and pitted below medulla. Cannula in tip of one lung, opposite lung tied off. Glottis open. Animal lost much blood and was breathing irregularly.

contractions or relaxation of the skeletal musculature are more rapid. The contractions are also too slow to be due to passive elastic rebound of the connective tissue of the lung. They are, however, very similar to the quick spontaneous contractions that are seen at times in the hypertonic frog lung after cutting the vagi or complete destruction of brain and spinal cord (fig. 7). The tracing in figure 3 illustrates the fact that these lung contractions may follow single respiratory movements, if the pause between two successive swallowings is of sufficient duration.

It would seem that these contractions of the lung musculature following the active inspiration or attempts at inspiration have not been seen by previous workers, except possibly Graham Brown (5). On

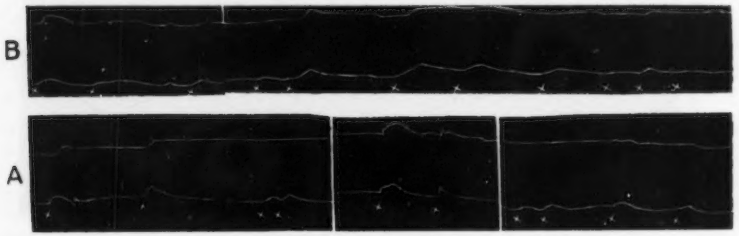

Fig. 4. Water manometer tracings of the contractions of the lung musculature in the frog (Rana pipiens) that follow upon the external respiratory movements. Spinal cord transected and destroyed below the medulla. Lungs isolated and cannulated (tip). Glottis closed with forceps so that there is no communication between the two lungs. *A*, upper tracing equals left lung; lower tracing equals right lung. *B*, same. *X* = pharyngeal respiration or attempt at swallowing air.

some of the tracings published by Brown there is an increased pressure in the lungs shortly before expiration, and Brown suggests that this is due to muscular contractions in the lungs.

The lung contractions do not depend on the change in tension on the lung tissues following a forceful inspiration. The glottis may be closed and the pressure in the lungs raised to that of the normal of 1 to 3 cm. of water, and in such preparations each attempt at respiration, single or a series, is followed by lung contractions (fig. 4). In preparations with the glottis closed the contractions are usually more prolonged than those seen in figures 2 and 3.

We are thus forced to the conclusion that in the frog the normal inspiratory movements lead to active contractions of the lung mus-

culature through associated innervation, either of the motor fibers to the lung or through central depression of the inhibitory fibers controlling a peripheral automatism.

b. Inhibition. In preparations with the glottis closed, and in intrapulmonic pressure approximately normal (1–3 cm. of water), *the active respiratory movements lower the intrapulmonic pressure*, evidently by inhibition of the lung muscle tonus (fig. 5). With the glottis closed no air can enter or leave the lungs. The respiratory movements of the throat and pharynx, especially if they are vigorous, induce slight fluctuations in the intrapulmonic pressure of equal rapidity with the pharyngeal movements. Vigorous movements of the head may induce,

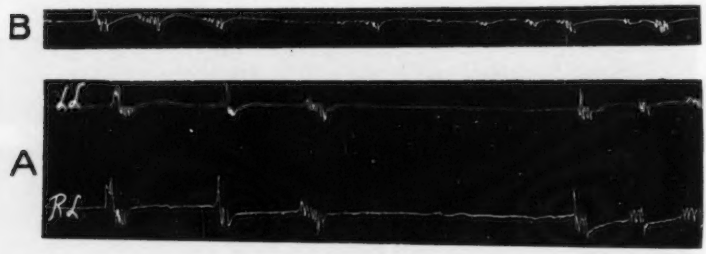

Fig. 5. Water manometer tracings of the intrapulmonic pressure in frogs (R. pipiens). Spinal cord cut and pithed below medulla. Cannula in tip of lung, and glottis closed (imperfectly) by cotton and collodion. *A*, simultaneous record from both lungs of the bull frog. *B*, record from lung of R. pipiens. Showing inhibition of the tonus of the lung musculature during the active respiratory movements. The rapid fluctuations (respiration) on the tracings are due to movements of larynx and head, and not to entrance and exit of air into the lungs.

possibly, stronger positive pressure in the closed lungs synchronously with these movements. It is possible but not probable that the lowering of lung muscle tonus during the rapid and rigorous swallowing movements are due to mechanical stimulation of the lung from this source, since the inhibition cannot be produced by similar fluctuations in intrapulmonic pressure artificially induced, and direct mechanical stimulation of the lungs when strong enough to have an effect causes contractions.

The return of the lung muscle tonus following the period of inhibition is quite similar to the lung contractions described in previous sections and illustrated in figures 2, 3 and 4. So far as we know, this inhibition of the lung musculature has not been noted by previous investigators.

The utility of the correlation is obvious, the relaxation of the lung musculature during inspiration being favorable to the filling of the lungs by the swallowing act.

THE PERIPHERAL MOTOR AUTOMATISM OF THE LUNGS AND THE INFLUENCE OF THE VAGI AND THE CERVICAL SYMPATHETIC NERVES ON THIS AUTOMATISM

1. In preparations with the glottis closed, lungs isolated from the influence of skeletal muscle contractions, section of the vago-sympathetic nerves in the neck, destruction of the medulla, or ligation of the base of the lungs *induces immediately a permanent hypertonus or incomplete tetanus of the lung neuro-muscular mechanism* (figs. 6, 8 and 9). By permanent, we refer, of course, only to the time of observation in these crucial experiments, that is, 2 hours. We found it difficult to maintain the preparation in good physiological condition over longer periods, especially with both lungs contracted down so that the lumen is completely obliterated thus preventing lung ventilation. The circulation also fails gradually.

The hypertonus of the lungs is usually at its maximum shortly after the isolation from the central nervous system, and there may be a gradual fall of the tonus during the observation period of 1 to 2 hours. This gradual fall is probably due to the failure of maintaining adequate circulation in the lungs, that is, to asphyxia.

This remarkable lung reaction is obtained in all frogs in good physiological condition, and the better the condition of the frog the stronger the lung tetanus on sections of the vagi. In frogs in poor condition (infected, starved or moribund from any cause) destruction of the medulla or vagi section causes little or no lung tetanus. In such preparations stimulation of the peripheral end of the cut vagi also fails to influence the lung tonus. Poor physiological conditions in the frog are evidently associated with lung atony, just as these conditions usually involve atony and absence of stomach rhythm in all species of animals so far studied.

We have occasionally found preparations showing marked lung tonus before section of the vagi or destruction of the medulla, that is, a tonus greater than that of normal respiration. We are inclined to ascribe this to the following causes: *a*, temporary depression of the medulla or nervous "shock" due to cutting the spinal cord, strong mechanical stimulation of many sensory nerves; *b*, direct mechanical injury to the

Fig. 6. Water manometer tracings of intrapulmonic pressure of frog (R. pipiens). Spinal cord transected and pithed below medulla. Frog fixed on dorsal side, abdomen laid open and lungs isolated from influence of skeletal muscles. Cannulae in tips of lungs. Glottis closed by forceps, shutting off at the same time air connections between the two lungs without interfering with lung circulation and lung innervation. *a*, Ligation of base of left lung (upper tracing). *b*, Section of right vagus (lower tracing = right lung). Signal line = base line pressure for right lung. Time, 5 seconds. Showing prolonged tetanus or tonus of lung neuro-musculature mechanism on severance of vagi nerves.

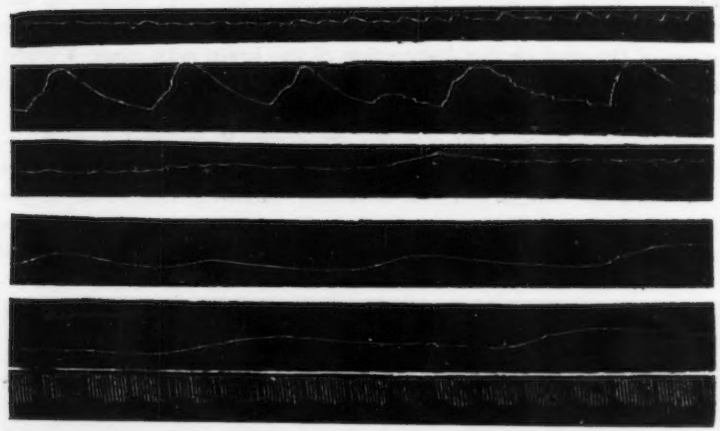

Fig. 7. Water manometer tracing of the intrapulmonic pressure in the frog (R. pipiens). Showing various types of peripheral automatism after isolation of the lungs from the central nervous system (section vago-sympathetic nerves or pithing of brain). Time, 5 seconds.

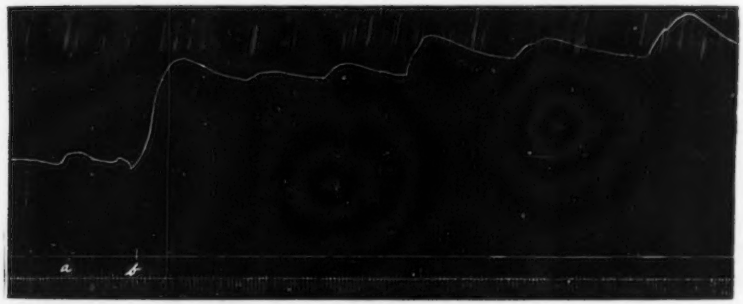

Fig. 8. Water manometer tracing of the intrapulmonic pressure in the frog's lung (R. pipiens), showing the incomplete tetany of the lung following destruction of the brain. Spinal cord cut and pithed below the medulla. Abdomen opened and lungs freed from influence of skeletal muscles. Cannula in tip of lung. Glottis closed by forceps, leaving vagi nerves and lung circulation intact. *a*, Transection of upper jaw. *b*, Crushing of brain (including medulla) by artery forceps. Time, 5 seconds.

lungs; *c*, partial asphyxia of medulla and lungs from failure of the circulation (unavoidable hemorrhage, etc.). In such preparations the section of the vagi or the destruction of the brain may cause a slight increase of the lung tonus (fig. 9). The lung of the bull frog passes into prolonged hypertonus on direct mechanical stimulation more readily than does the lung of the common grass frog (R. pipiens).

In our most vigorous preparation the lung tetanus following isolation from the central nervous system is extreme, that is, all of the air is driven out of the lung cavity into the water manometer and the lung cavity is completely obliterated. Even the traces of mucus are

Fig. 9. Water manometer record of the intrapulmonic pressure in the frog's lung, showing moderate tetanus or tonus of the lung on destruction of the brain. Spinal cord cut and pithed below the medulla; frog fixed on dorsal side, and lungs isolated from influence of skeletal musculature. Cannula in tip of lungs; glottis closed by forceps, also shutting off air communication between the two lungs. Signal = crushing of brain with forceps. Signal line = the zero line of water pressure for right lung (lower tracing). Time, 5 seconds.

forced into the cannula in the tip of the lungs. The maximum intrapulmonic pressure thus developed is from 6 to 10 cm. of water above the pressure existing during normal respiration, that is, a total pressure of from 7 to 12 cm. of water. This is not the maximum pressure the lung tetanus is capable of developing. If the lungs are connected with a mercury manometer so that some air still remains in the lung cavity, even under the maximum lung tetanus following vagi section, the intrapulmonic pressure rises to the surprising height of 20 to 40 mm. Hg. (25–50 cm. water).

2. The nature of the peripheral lung tetanus. In most of our preparations the water manometer tracings of the lung tonus following isolation

of the lungs from the central nervous system show a straight line indicating a continuous tonic or complete tetanic contraction (fig. 9). The more vigorous preparations exhibit various types of rhythmic contractions superimposed on the continuous hypertonus, at least during the first 15 to 60 minutes following the lung isolation. But even in these preparations the rhythm fails before the complete failure of the tonic state of contraction in later stages of the record. It would thus seem that the appearance of rhythm on the hypertonic state of the lung is a question of physiological condition of the lung motor mechanism.

The usual type of the rhythmic lung contractions is shown in figures 6 and 8, that is, a slow rhythm, the contraction and relaxation requiring 2 to 3 minutes. In general, the more vigorous the preparation the more rapid the rhythmic contractions. In other preparations contractions last only 20 to 30 seconds, and occasionally this faster rhythm may be superimposed on the slower rhythm (fig. 7), both rhythms being in turn superimposed on the continuous hypertonus. Whether these varying types of contractions involve different musculatures cannot be made out by the present method of experimentation. The continuous hypertonus as well as the strong slow rhythm seem to involve the whole lung. The more rapid rhythm is too feeble to be detected by direct inspection of the lung. Nothing that could be interpreted as peristaltic contractions similar to those exhibited by other visceral structures has so far been noted by us, although our water manometer tracings of the lung hypertonus as slow rhythmic contractions suggest many points of similarity to the contractions of the empty stomach as recorded by the balloon method. This may be of significance in connection with the fact that the lung is a diverticulum from the foregut (esophagus).

It appears that this striking lung tetanus or hypertonus following isolation of the lungs from the central nervous system has not been observed by previous investigators, evidently because none have sectioned the vagi after closing the glottis under conditions permitting recording the neuro-muscular tonus of the lungs. Several workers have studied the influence of vagi section on the external respiratory movements of the frog. Mochi states that the lungs remain permanently collapsed and empty when the medulla and vagi are left intact but all the brain anterior to the medulla removed. This is probably an error. Langendorff claims, at least, that the external respiratory movements persist after removal of the brain anterior to the medulla. According to Martin (16) and Mochi (17), (18), (19), stimulation of the optic

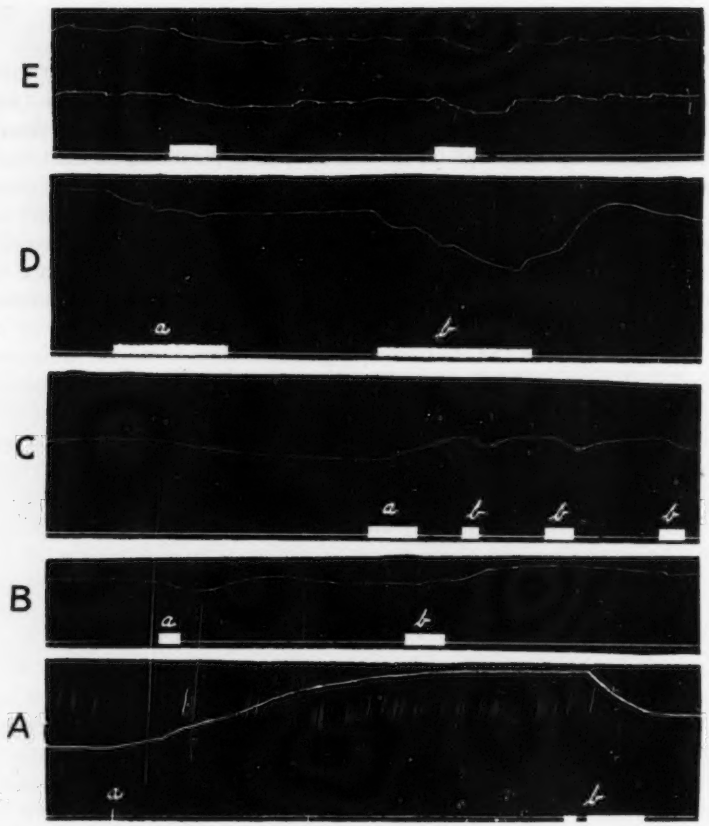

Fig. 10. Water manometer tracings of the intrapulmonic pressure in the frog (R. pipiens). Spinal cord cut and destroyed below medulla; lungs isolated and cannula fixed in tip of lungs. Glottis closed.

A: Right lung: *a*, section of vago-sympathetic nerve; *b*, stimulation of peripheral end of cut nerve with moderately strong tetanizing current.

B: Left lung: left vago-sympathetic nerve sectioned. Stimulation of peripheral end of cut nerve with weak, *a*, and strong, *b*, tetanizing current.

C: Left lung, after section of left vago-sympathetic stimulation of peripheral end of cut nerve with very strong, *a*, and moderately strong, *b*, tetanizing current.

D: Left lung, in very strong tonus after section of left vago-sympathetic nerve. Stimulation of the peripheral end of the cut nerve with weak, *a*, and strong, *b*, tetanizing current.

E: Upper tracing = left lung; lower tracing = right lung. Signal = stimulation of the peripheral end of the cut nerves. Showing inhibitions of lung tonus and contractions on stimulation of the peripheral end of the vago-sympathetic nerve.

lobes accelerates the respiratory movements. This may indicate a subsidiary respiratory center anterior to the medulla. At any rate mere decerebration does not induce lung tetanus in our experience.

Martin (16) states that destruction of the brain and spinal cord leaves the lungs entirely empty of air, but he does not make out or recognize that this is due to a persistent lung tetanus. Babak (2) quotes a number of authors as having shown that after vagi section or lung extirpation the frogs swallow air, periodically, into the stomach, and the air may actually escape by the cloaca. Berti and Marzemin (4) state that section of the vagi peripheral to the superior laryngeal branch results in irregular attempts at lung respiration on elevation of the temperature. Nikolides (20), (21) states that vagi section slows the respiratory movements making them at the same time irregular and stronger. Heinemann (9), one of the earliest observers, states that section of both vagi leads in the course of several days to such abnormal filling of the lungs that some of the viscera are pushed out through the cloaca. But when he opened the abdomen of these frogs the lungs were found collapsed or only partly filled. It is possible that Heinemann's frogs actually swallowed air into the stomach and intestines because of persistently constricted lungs. Soprana (24) states that vagotomized frogs breathe slower and deeper, but die more quickly from asphyxia on elevation of the temperature. According to Pari (22), the vagotomized frog is unable to fill the lungs, the lungs remain collapsed for weeks, and the air is forced into the stomach. It is possible that Pari's permanently collapsed lungs were in reality in a constricted state. But the method of observation did not suffice to record the fact.

It is certain that the force of the air swallowing would fail to cause air to enter the lungs against the maximum state of lung contraction found by us after section of the vagi nerves. But we do not know how long this hypertonus persists in the surviving animal, as all our experiments to date have been crucial. And even if the hypertonus, tonus or tetanus remained as long as the frog continued in good condition, failure of lung respiration may soon operate to place the frog below par in general, in which case there may be depression of the peripheral lung automatism already noted by us in animals in poor condition. The process of physiological readjustment of the peripheral lung motor mechanism may also come into play, similar to the readjustment that gradually takes place in the case of the heart and the respiratory center in the medulla after vagi section.

3. The action of the vagi and the cervical sympathetic nerves on the lung motor mechanism. a. The cervical sympathetic nerves. Section of the cervical sympathetic before its union with the vagus has no effect on the lung tonus (fig. 11), in marked contrast to the effects produced by section of the combined vago-sympathetic nerve. Electrical stimulation of the peripheral end of the cut cervical sympathetic causes slight contraction of the lung on the same side. It is difficult to stimulate the cervical sympathetic nerves, under the conditions of our experiments, and at the same time avoid escape of the current to the vagus

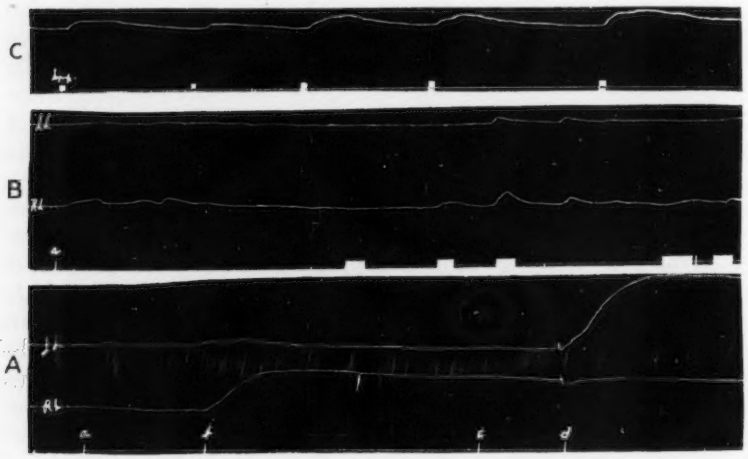

Fig. 11. Water manometer tracings of the intrapulmonic pressure in the frog's lungs (R. pipiens). Frogs decerebrated, animals fixed on dorsal side, lungs isolated from influence of skeletal muscle contraction. Cannula in tip of lungs. Glottis closed.

A: Upper tracing, left lung; lower, right lung. *a*, section right cervical sympathetic; *b*, section of right vagus; *c*, section of left cervical sympathetic; *d*, section of left vagus.

B: Upper racing, left lung; lower, right lung. *a*, section of right cervical sympathetic nerve; signal, stimulation of peripheral end of right cervical sympathetic. In this preparation the left cervical sympathetic and both vagi were intact.

C: Record from left lung, showing lung contractions on stimulation of the peripheral end of the cervical sympathetic nerve with strong tetanizing current, the vagus being intact.

Showing motor fibers to the lungs in the cervical sympathetic nerve, but no effect on lung tonus from section of these motor fibers.

ganglion, or to other sensory nerves, thus inducing reflex effects. Our usual technique was to section the large brachial nerve close to the vertebral column, taking care not to injure the slender sympathetic trunk passing under it; also section the root of the hypoglossal, and after again sectioning these nerves peripherally, handle the cervical sympathetic by the stump of the brachial to which it is attached. We also made it a point to apply the fine pointed electrodes to the cervical sympathetic trunk at least 3 mm. distant from the vagus root. But even with the best of precautions escape of current to adjacent structures could not always be prevented. And we are inclined to explain the *bilateral* lung effects produced by the stimulation of one sympathetic (fig. 11, *B*) as due to escape, and consequent reflexes. It is to be noted further that it requires relatively strong tetanizing currents applied to the sympathetic trunk to secure the lung contractions. Inhibitory effects on the lung were never obtained from the cervical sympathetic nerves.

Our conclusion is that the cervical sympathetic trunk carries motor (but no inhibitory) fibers to the lungs via the vagi. Under the conditions of our experiments the section of these motor fibers has no effect on the lung tonus, showing that this motor mechanism is not in tonic activity, and that the section of the nerves is not a sufficient stimulus for even a transient contraction.

b. The vagi nerves. We have seen that section of the vagus induces permanent hypertonus in the lung of the same side. Stimulation of the peripheral end of the cut vagus with a tetanizing current causes an inhibition of this tonus followed by a return to the former state. The vagus stimulation is thus able to completely abolish (temporarily) the tonus induced by the vagi section. In the preparations showing no lung hypertonus on vagi section owing to peripheral lung atony vagus stimulation usually causes no lung inhibition.

In several such preparations we observed that the vagi also failed to influence the heart rhythm. We can state that the failure of the vagi to act in the normal manner on the lungs and heart in these preparations was not due to mechanical injury to the vagi or to the heart and lungs. The significance of this coincidence requires further investigation. It is well known to laboratory workers in physiology that one frequently encounters frogs in which the vagi stimulation fails to influence the heart. This inhibitory action of the vagus on the lung is obtained with the minimum and up to relatively strong tetanizing currents. The stronger stimuli produce at times motor after-effects,

and very strong tetanizing currents may produce contraction only or a brief initial contraction followed by inhibition. This latter result was obtained by strong stimuli, especially in preparations showing less than the maximum lung tonus following vagus section.

Stimulation of the peripheral vagus inhibits not only the tonus but also the spontaneous rhythmic contractions that may be superimposed on the lung hypertonus following isolation from the central nervous system (fig. 10, *E*).

It is thus clear that the vagi and the cervical sympathetic nerves in the frog bear the same physiological relations to the lungs as they do to the heart, that is, motor fibers in the latter and inhibitory fibers in the former to both organs. We shall show later in the section on the action of drugs on the lungs, that some of the motor fibers to the lungs are true vagi fibers, and do not belong to the cervical sympathetic complex.

4. The peripheral lung automatism. We are now in position to analyze more definitely the origin of the motor hypertonus of the lungs after vagi section. It is not due to temporary stimulation of motor fibers. We have shown that section of the sympathetic nerve fibers has no effect. There are some motor fibers to the lungs of pure vagus origin. But cutting of these fibers produces no effect on the lungs, after previous paralysis of the inhibitory vagi fibers by large doses of nicotine. It is not due to mechanical trauma to the lungs. Ligation of the base of the lung may induce lung tetanus by direct trauma or by asphyxia, lung circulation being cut off. But stopping the circulation by excising the heart does not cause lung tetanus, and section of the vagi is done without touching the lungs or the adjacent structures. Moreover, the indirect mechanical disturbance of the pharynx and the base of the lungs is much greater from isolation of the vagi or the cervical sympathetic nerves, and these latter procedures do not bring on lung tetanus. The lung hypertonus is not an asphyxia phenomenon. Excessive lung ventilation, normal or artificial, will not prevent or abort it, if the vagi are sectioned or the brain destroyed. The lung hypermotility is not a temporary motor reflex state induced via the medulla by the powerful afferent impulses induced by the extensive operative trauma, for the lung is found collapsed and maximally contracted in frogs with the brain destroyed, without previous operative injury of any kind, and we know of no other reflex state that may persist for hours after lesion of the reflex path. It is not unlikely, however, that any condition inducing a peripheral lung hypertonus of a degree interfering with the

respiratory functions of the lungs would augment the inhibitory action of the medulla on the lungs, probably through sensory fibers from the pulmonary branches of the vagi, a mechanism analogous to that of the depressor nerves and the cardio-inhibitory center.

In the experiments with pithing the medulla we at times obtained a slight temporary inhibition of the lung tonus prior to the typical lung tetanus. The temporary inhibition is evidently due to a transient stimulation of the medulla inhibitory center by the act of destruction. It is not probable that the subsequent lung hypertonus is due to a more lasting traumatic stimulation of the motor fibers, analogous to the effect produced on the heart by strong stimulation of the two sets of fibers in the vagi nerve trunks. This possibility is disproved by the fact that nicotine paralyzes the lung inhibitory nerve mechanism, leaving the motor nerve mechanism intact, and nicotine causes a lung tetanus which is not augmented by subsequent destruction of the medulla or section of the vagi.

Hence we must conclude that in the frog the vagi inhibitory fibers to the lungs are in constant or tonic activity, holding the peripheral motor automatism in check, and that on removal of this check the lungs go into a persistent tetanus or hypertonus. In other words, the lungs of the frog behave like the heart of many species of animals on section of the vagi; the heart beats faster, the lungs become hypertonic to a degree that nullifies their function.

These observations place the lung of the frog in the same category as the heart and the alimentary tract as regards independent peripheral motor automatism. In all these structures we have the same motor tissues, viz., nerve cells, nerve plexuses and musculature. We are therefore confronted by the same problems as regards the nature of the mechanism of the lung automatism that have engaged the attention of the physiologists in connection with the heart and the gut. A question of equal importance is the persistence or modification of this primitive lung automatism, in health and disease, in other groups of lunged animals.

CHANGES IN INTRAPULMONIC PRESSURE AS A RESULT OF THE STIMULATION OF VARIOUS AFFERENT NERVES

No investigations have been made on reflexes into the lung musculature. Most of the previous workers have been engaged in a study of the external respiratory phenomena of the frog. Wendenski (26) noted

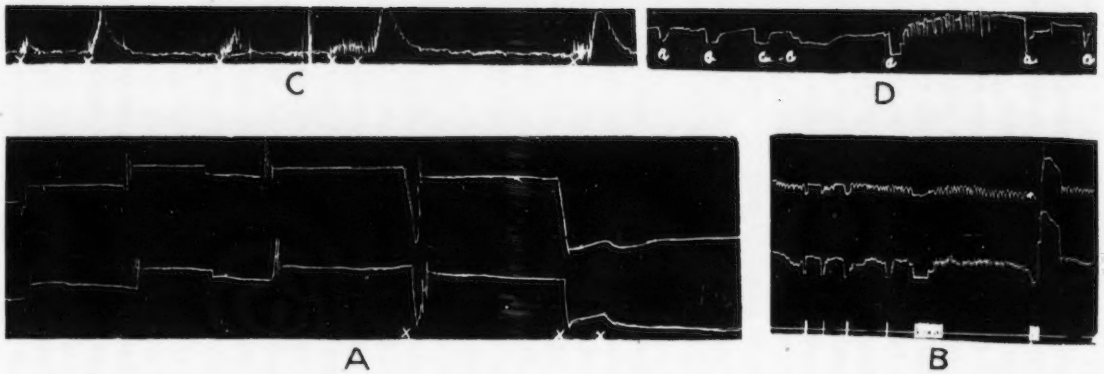

Fig. 12. Water manometer tracings of the intrapulmonic pressure in frogs. Glottis free. Cannula in tip of lungs. Tracings *A*, *B*, *C*, frogs decerebrated. Tracing D, spinal cord cut and destroyed below medulla leaving the brain intact. *A*, Bull frog. Upper tracing, left lung; lower, right lung. *X*, rubbing skin of hind leg.

B: (R. pipiens) upper record, left lung; lower, right lung. Signal, electrical stimulation of the urinary bladder.

C: X = gentle stroking of skin of hind leg, resulting in accelerated inspiration.

D: a = approach of finger on any moving object within the frog's visual field, followed by inhibition in a state of complete expiration or collapse of lungs.

"expiratory tetany" following weak stimulation of sensory fibers in the vagi. His method as well as that of other workers was not designed to note actual contractions of the lung itself, since in every case previous experimenters worked with an open glottis. However, tracings 15 and 16 of this article, taken from doubly vagotomized frogs, show very slight inspiratory and expiratory excursions of the flanks and long tonus variations obviously due to the strong tonus of the lung and the tonus contractions in the lung as seen by us.

Sensory stimulation of any sort, be it electrical or mechanical, has a powerful effect on the external respiration of the frog by either reducing the intrapulmonic pressure by reflex opening of the glottis, or if the latter is closed by hemostat or pressure by vaselinized cotton at the time of application of stimulus, by reflex lung contraction which will cause the intrapulmonic pressure to rise.

Figure 12 *A* shows at *X* the sudden opening of the glottis in decerebrated bull frog, holding air under considerable pressure, following gentle stroking of the skin of the hind leg. The first part of the tracing shows voluntary respirations (swallowing of air) with maintenance of high intrapulmonic pressure. The prompt collapse of the lung is followed immediately by marked respiratory effects which raised the intrapulmonic pressure to its original level. Stimulation of the skin in another preparation similarly prepared (fig. 12 *C*) not only increased the volume of the respiratory gulps, which were occurring regularly and continuously prior to the stimulation, but induced the animal in every instance to fill the lungs to the maximum capacity in the fashion described in the second section of this paper.

Figure 12 *B* shows a similar collapse of the lungs in Rana pipiens due to opening of the glottis following electrical stimulation of the urinary bladder with a moderately strong tetanizing current.

Tracing *D*, figure 12, was obtained from a frog with brain intact. In this animal the simple approach of the finger or person at *a* led to collapsed lung followed by more or less marked efforts at refilling.

In all tracings reproduced in figure 12 the glottis was open. These preparations, therefore, were not favorable for a study of the pulmonary activity itself (lung contractions or inhibitions) following the stimulation of various afferent nerves. In order to maintain the volume of air constant we closed the glottis by a mosquito forceps or vaselinized cotton and raised the intrapulmonary pressure to a point maintained by the animal under normal conditions and then noted the effect of the stimulation of the afferent nerves.

The ligation of the vagus on one side (mechanical stimulation) in many instances induced reflex contraction of the opposite lung. In the present state of our knowledge it is impossible to state whether record of such a contraction as shown in figure 13 *A* at *X* is due to reflex stimulation of the lung through the motor fibers of the vagus or due to a temporary inhibition of the tonic inhibitory control over the lung via the vagi, leaving the peripheral automatic mechanism in the lung unchecked. A possible answer to this question might be obtained by noting the effect of such stimulation in animals in which the tonic inhibitory mechanism has been previously paralyzed by nicotine. If under these conditions stimulation of the sensory nerves yields the same results the recorded contraction is the result, not of a temporary inhibition of the tonic inhibitory mechanism, but due to the reflex stimulation of the pulmonary motor fibers through the vagi (and sympathetics). Consideration of the law of reciprocal innervation would suggest that under normal conditions both mechanisms are involved in the phenomenon whose graphic record is that of a rise in intrapulmonic pressure.

Irrespective of the mechanism or mechanisms involved in ultimately effecting contractions of the lungs, we can confidently say that stimulation of the skin of the upper mandible, mild mechanical irritation of the anterior nares, mechanical stimulation of the bladder and cloaca, or electrical stimulation of the urinary bladder, mesentery, small intestine, pyloric end of stomach, esophagus and central end of the brachial nerve effect reflex contractions of the lung. These points are shown individually in figure 13 and figure 14, which with the accompanying legends are self-explanatory. The mode of preparation of the animals used in both series is virtually the same with this exception: In the preparations, records of which are illustrated in figure 13, the glottis was clamped by means of a mosquito forceps; in the tracings reproduced in figure 14 the glottis was kept occluded by pressure over it by a piece of vaselinized cotton.

Of these records two deserve particular attention. In figure 13, *D*, is recorded the powerful reflex lung contractions obtained by closure of the glottis by a hemostat. A decided inhibition preceded the contraction.

Tracing *E* in the same figure shows an unusually strong reflex contraction of both lungs as a result of crushing the skin of the lower mandible.

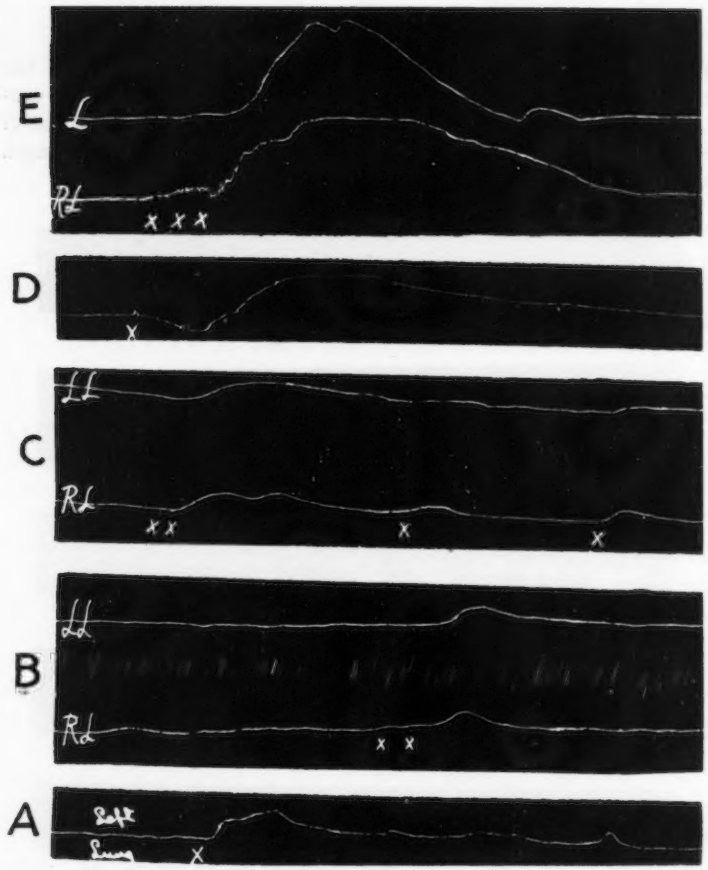

Fig. 13. Water manometer tracing of intrapulmonic pressure in the frog (R. pipiens). Spinal cord cut and destroyed below medulla. Cannula in tip of lungs, abdomen opened and the lungs isolated from abdominal and shoulder musculature. Glottis closed by clamp except in tracing *D*.

A: Left lung; *x*, ligation of right vago-sympathtic nerve, showing reflex contraction of left lung on vagus stimulation.

B: Upper tracing, left lung; lower, right lung. *X*, mechanical stimulation of the skin of the upper mandible, showing reflex lung contraction.

C Upper tracing, left lung; lower, right lung. *XX*, mechanical stimulation of the nares; *X*, mechanical stimulation of the cornea, showing reflex lung contractions.

D: X, closure of the glottis with artery forceps, showing temporary reflex inhibition of lung tonus followed by strong contraction.

E: Upper tracing, left lung; lower equals right lung. *XXX*, crushing skin of lower mandible, showing exceptionally strong reflex lung contractions.

In this series of experiments stimulation of the fundic end of the stomach (fig. 14, *D*, "*c*") with the electrical current yielded no reflex contraction of the lung at a time when stimulation of the pyloric end of the stomach and cardiac region of the esophagus with the same strength of current gave uniformly striking results.

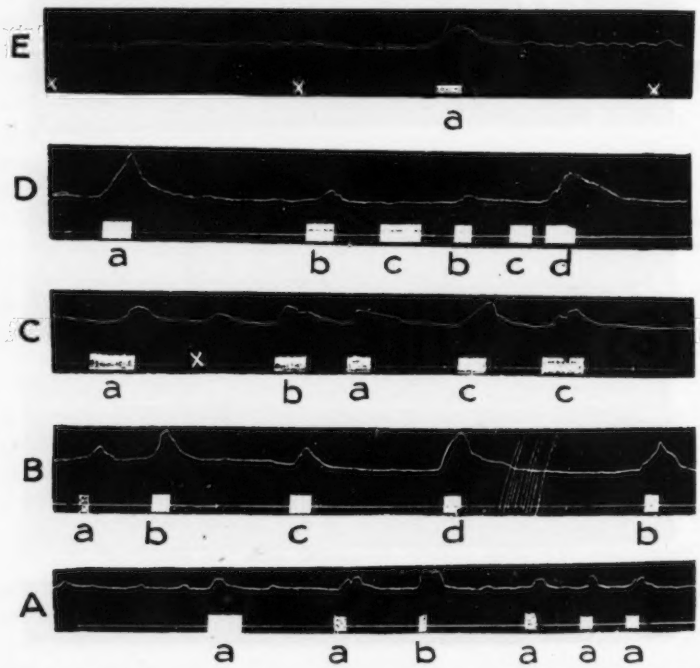

Fig. 14. Water manometer tracings of the intrapulmonic pressure in the frog's lung, showing reflex contractions of the lung musculature. Frogs decerebrated. Abdomen opened, lungs isolated from influence of skeletal muscle contractions. Cannula in tip of lungs, and glottis closed with a plug of vaselined cotton pushed into the pharynx.

A: *a*, mechanical stimulation (stroking) skin of hind leg; *b*, pinching toes of hind leg.

B: *a*, mechanical stimulation of urinary bladder; *b*, electrical stimulation of urinary bladder; *c*, mechanical stimulation of cloaca.

C: Electrical stimulation; *a*, large intestine; *b*, mesentery; *c*, small intestine.

D: Electrical stimulation; *a*, small intestine; *b*, pyloric end of stomach; *c*, fundus of stomach; *d*, esophagus (cardiac region).

E: *a*, electrical stimulation of central end of brachial nerve plexus. *X*, spontaneous respiration (quick up stroke).

In a summarizing sentence we might therefore state that the stimulation of every sensory nerve (afferent visceral or cutaneous) gives rise reflexly to lung contractions.

THE ACTION OF CERTAIN DRUGS ON THE MOTOR MECHANISM OF THE LUNG

Our interest in the action of drugs on the neuro-muscular mechanism of frog's lung had its inception during our study of the physiological action of the vagus on the lung musculature following electrical stimulation of the nerve. Such stimulation gave at outset variable results until we noted that the effects depended to some extent on the strength of the tetanizing current employed, as noted above. At any rate, we had good reason to suspect that the vagus carried both motor and inhibitory fibers to the lung motor mechanism. At this juncture it occurred to us that the use of drugs might be helpful in clarifying the situation.

Nicotine. Mindful of the action of nicotine in abolishing the inhibitory effect of vagus stimulation on the heart without affecting the motor action, we assumed that the drug might act similarly with respect to the inhibitory fibers in the vagus to the lung. If this were so, electrical stimulation of the vagus following the injection of nicotine might give clearer evidence of motor fibers in this nerve than before nicotinization. Since, furthermore, the paralysis of the ganglion cells in the course of the inhibitory fibers to the heart effected by this drug is preceded most commonly by *stimulation*, the same effect might be anticipated in the case of the inhibitory fibers to the lungs. If this were true nicotine ought to cause, on injection, an inhibition similar to, if not identical with, the inhibition of the heart commonly observed as the marked effect of stimulation of the vagus before the injection of the drug, especially if the tonic central inhibitory control exercised over the lungs via the vagi had been abolished by either sectioning of the nerves or destruction of the medulla.

The results obtained exceeded our expectations. Inspection of figure 15 (at *g*) shows that 1 mgm. nicotine when intravenously injected effects a pulmonary inhibition in the lungs released from the tonic inhibitory influence of vagus by section of these nerves (at *a* and *b*) which compares favorably with the inhibition obtained by previously stimulating the nerves with a tetanizing current of moderate intensity (see *e* and *f*).

If, on the other hand, the nicotine is injected intravenously in an animal following ligation and section of but one vagus, as in figure 16,

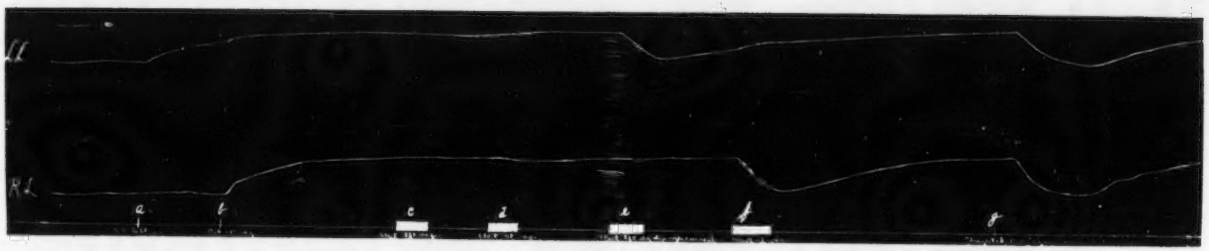

Fig. 15. Water manometer tracings of the intrapulmonic pressure in the frog's lung (R. pipiens). Spinal cord sectioned and destroyed below medulla. Cannula in tip of lungs. Lungs isolated from influence of skeletal musculature. Lower tracing, right lung; upper tracing, left lung. Glottis closed. *a*, Section of left vagus; *b*, section of right vagus; *c* and *d*, stimulation of left and right vagus with very weak tetanizing current; *e* and *f*, stimulation of left and right vagi with stronger tetanizing current; *g*, injection of 1 mgm. nicotine in 5 cc. Ringer's solution into abdominal vein.

Showing nicotine inhibitions of lung muscle tonus identical with the vagi inhibitions.

the immediate effect on the lungs is an inhibition of the tonus of the lung which has been denervated, and an escape from the tonic inhibitory influence exercised by the vagus on the lung which is still connected with its center, as at *c*, where the left lung (upper tracing) shows a rise in tonus occurring in the course of an inhibiton of the right lung, the latter comparing favorably with the inhibition effected by previous stimulation of the vagus (*b*). In this experiment the left vagus has been cut *physiologically* by the drug. If at the time of this drug cutting the vagus through central action is exercising its maximum inhibition on the lung, the effect of the primary stimulating action of the drug would not appear since the lung at the time of drug stimulation is already under maximum inhibitory control. As a matter of fact, in the majority of preparations this is apparently the case, the drug nicotine simply releasing the peripheral automatic mechanism from the maximum tonic inhibitory effect of the center through the vagus. This release is certainly complete for section of the vagus to this lung is without further effect on its tonus. This latter fact would furthermore indicate that the more or less prompt rise of intrapulmonary pressure following section of the vagus without nicotine was due, not to the mechanical stimulation of the motor fibers contained in this nerve, but to the removal of the tonic inhibitory control. That the vagus nerve contains such motor fibers can be shown very satisfactorily in any preparation that has been nicotinized. In figure 16 electrical stimulation of the nerves after nicotine (as at *g*, right vagus, and *h*, left vagus) gives now marked contraction of the lung instead of the usual inhibition before nicotine (*b*).

Figure 17, *A*, is offered as another example of this phenomenon. Following the release of the left lung from tonic inhibitory control exercised over it through central vagal control by section of the left vagus at *a*, 2 mgm. nicotine were injected at *b* with the result that the right lung was now released from its inhibition by the "cutting" action of the drug and the left lung was inhibited by the primary stimulation action of the drug on the inhibitory mechanism of the left lung. Figure 17, *B*, shows the change in effect as a result of electrical stimulation of the vagus nerve following the injection of nicotine. In this experiment stimulation of the vagus at *b* caused pronounced inhibition. The injection of nicotine at *c* was followed by the usual inhibition in the lung which has been released from the tonic inhibitory control by section of the nerve at *a*. Subsequent, however, to this nicotinization, electrical stimulation at *d* caused marked contraction of the lung instead of inhibition.

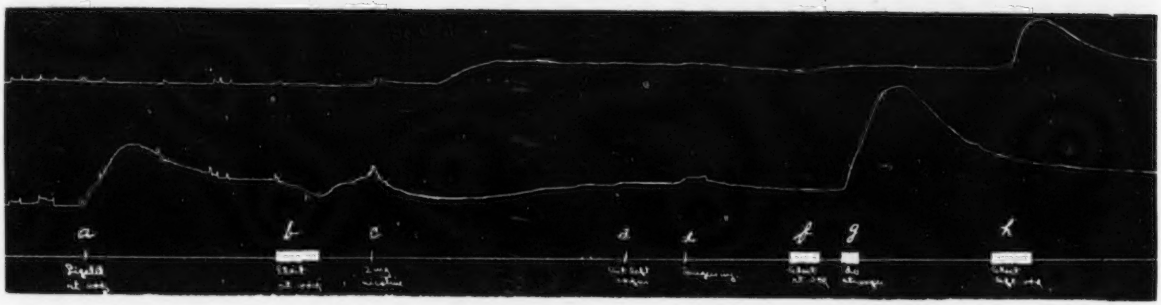

Fig. 16. Water manometer tracings of the intrapulmonic pressure in the frog's lung (R. pipiens). Spinal cord cut and destroyed below medulla; glottis closed; cannula in tip of lungs. Lungs isolated from influence of skeletal musculature. The quick up-strokes on tracings to the left of *C* are due to movements of the larynx in the spontaneous respiratory movements. Upper tracing, left lung; lower, right lung. *a*, Ligation of right vagus; *b*, electrical stimulation of right vagus; *c*, injection of 2 mgm. nicotine in 10 cc. Ringer's solution into abdominal vein; *d*, ligation, left vagus; *e*, injection of 10 cc. Ringer's solution into abdominal vein; *f* and *g*, stimulation of right vagus with weak and strong tetanizing current; *h*, stimulation of left vagus with tetanizing current.

These tracings show the primary inhibitory action of vagus stimulation and nicotine on the lung hypertonus following section of the vagi, the paralysis of inhibitory fibers of the vagi by nicotine thus permitting the full development of peripheral lung tonus, and the reversal of the inhibitory action of the vagosympathetic action after nicotine.

The results are uniformly clear-cut and decisive. By means of this drug nicotine it is possible to differentiate between two types of efferent pulmonary fibers occurring in the vagus, i.e., inhibitory fibers and motor fibers. The former exercise in the normal frog a powerful inhibitory control over the lung and are either more numerous or more readily susceptible to electrical stimulation than the motor fibers. It is only after the abolition of the inhibitory control of the lung by nicotine that

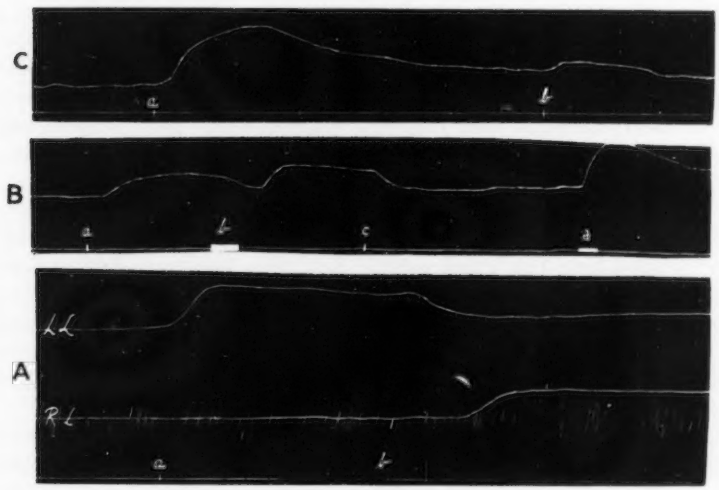

Fig. 17. Water manometer tracings of the intrapulmonic pressure in the frog (R. pipiens). Spinal cord cut and destroyed below the medulla. Glottis closed. Cannula in tip of lungs. Lungs isolated from influence by skeletal musculature.

A: Lower tracing, right lung; upper, left lung. *a*, Ligation of left vagus; *b*, injection of 2 mgm. nicotine in 10 cc. Ringer's solution into abdominal vein. Showing abolition of the tonic vagus inhibition of the lung neuro-muscular mechanism by nicotine.

B: Tracing from right lung. *a*, Ligation of right vagus; *b*, stimulation of peripheral end of right vagus with weak tetanizing current; *c*, injection of 2 mgm. nicotine in 10 cc. Ringer's solution into abdominal vein; *d*, stimulation of peripheral end of right vagus with same strength of tetanizing current as at *b*. Showing inhibition of lung tonus and paralysis of the vagi inhibitory fibers by nicotine.

C: Tracing from left lung. *a*, Injection of 5 mgm. nicotine in 5 cc. of Ringer's solution into the heart; *b*, injection of 1 cc. 1–1000 histamine into the heart. Showing stimulation of the lung by large doses of nicotine and stimulation by histamine after paralysis of the inhibitory nerves by nicotine.

electrical stimulation yields a more or less marked motor effect. Nor are these motor fibers of sympathetic origin running in the trunk of the vagus; for, granting the presence of some motor fibers in this nerve to the lung, the motor response on stimulation of the sympathetic after nicotine is smaller in a given animal than the response from the vagus itself, as noted earlier in the paper. In short, the vagus nerve contains two sets of fibers to the pulmonary motor mechanism of which the inhibitory exerts a tonic predominant control; the motor fibers are apparently not in a state of tonic activity for sectioning of the vagus after cutting the inhibitory fibers in this nerve by nicotine has no further effect on the intrapulmonic pressure. It would appear on the basis of our pharmacological studies that the inhibitory fibers of the vagus have interpolated in their course to the automatic tissues nerve cells on which the drug acts; the motor fibers on the other hand run directly to the automatic tissue.

Large doses of nicotine. Whereas the constant effect of the intravenous injection of small doses (2 mgm.) of nicotine in the previously denervated lung (by vagotomy) is inhibition, injection of large doses (5 mgm. or more) causes pronounced contraction of the lung. This is well shown in figure 17, *C*, where the injection of 5 mgm. caused more or less abrupt contraction followed by slow relaxation. It is probable that the nicotine in this dosage acts as a direct stimulant to the smooth musculature.

Atropine. This drug, even when given in large doses, does not paralyze the endings of the inhibitory fibers to the lungs as it paralyzes the cardio-inhibitory nerve endings. Figure 18, *A*, is a tracing from a frog which had received 1 cc. of a 0.1 per cent solution of atropine sulfate 45 minutes previous to experimentation. Pithing of the brain at *a* was followed by the typical escape of the lung from tonic central inhibitory control. The failure of atropine to paralyze the inhibitory nerve ending in the lung is shown further by the fact that even mechanical stimulation of the nerve at *b* gave powerful inhibition, as did electrical stimulation at *c*. As an after-effect of mechanical or electrical stimulation of the nerve there were pronounced motor effects. The results obtained from the right lung of another frog were somewhat different. Here (fig. 17, *B*) ligation of the vagus caused the usual escape of the lung from the inhibition. On the other hand, mechanical stimulation due to handling of the vagus nerve (at *b*) was followed by contraction. Stimulation of this nerve at *c* with a mild tetanizing current was in this instance followed by contraction instead of the usual inhibition.

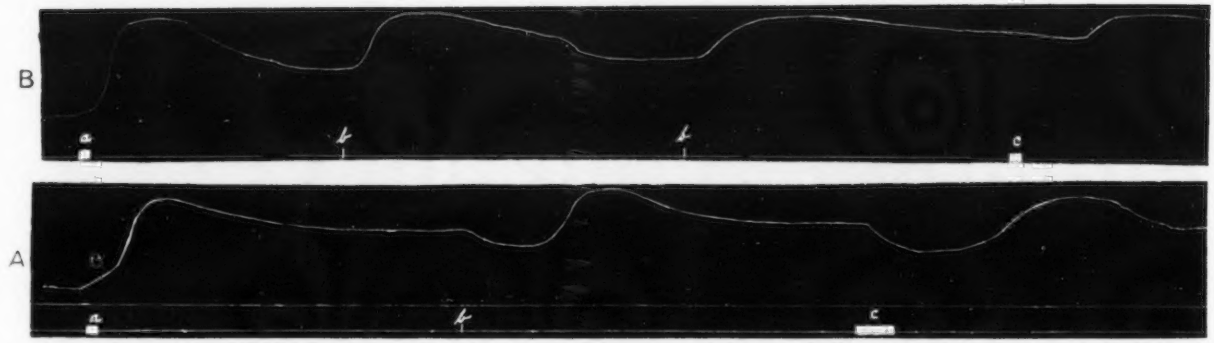

Fig. 18. Water manometer tracings of the intrapulmonic pressure in the frog (R. pipiens). Spinal cord cut and destroyed below medulla. Cannula in tip of lungs. Glottis closed. Lungs isolated from influence of skeletal muscle contractions.

One cubic centimeter of 0.1 per cent solution of atropin sulphate injected into the dorsal lymph sac 45 minutes before animals were prepared for the experiments.

A: Left lung; *a*, brain pith; *b*, mechanical stimulation (pulling) of left vagus; *c*, electrical stimulation of left vagus.

B: Right lung; *a*, ligation right vagus; *b*, mechanical stimulation of right vagus; *c*, electrical stimulation of right vagus with weak tetanizing current. Showing persistence of vagi inhibitory influence on the lung with exaggeration of the motor after effects after atropin in sufficient quantities to paralyze the cardiac vagi fibers.

To meet the objection that in these cases the dosage was too small and that the effects of the drug had worn off before experimentation was begun, we prepared another frog as follows: After decerebration, insertion of the cannula into the tips of both lungs, and clamping of the glottis, we sectioned the left vagus and obtained the usual escape of the lung from the tonic central inhibition control. Stimulation of this nerve gave the usual inhibition. The intravenous injection of $\frac{1}{2}$ mgm. atropine did not effect the result of stimulation of the vagus. We now injected within 1 hour's time in successive doses, 1, 2 and 4 mgm. atropine sulphate. These injections did not change the usual reaction obtained from stimulation of the peripheral vagus. Following the injection of the last 4 mgm., the right lung escaped from central inhibitory control in a manner indistinguishable from section of its vagus nerve. Apparently this huge dose of atropine paralyzed the center, for section of the right vagus was without further effect. Stimulation of its peripheral end, however, yielded even now inhibition followed by contraction of the lung, indicating that the chief action on the lung of atropine in huge doses is not peripheral. Since decided motor after-effects result from stimulation of vagus to the lung in a heavily atropinized frog, we might conclude that the drug likewise has *some effect* in paralyzing the inhibitory nerve endings unless we assume that it sensitizes the motor nerve endings in the vagus. At any rate the peripheral action of atropine and nicotine are quantitatively decidedly different if one compares the dosage in milligrams and the results effected thereby.

Both drugs paralyze the center and likewise act on the peripheral mechanism. Nicotine accomplishes both results quickly and decisively in smaller doses, while atropine acts on the center only in large doses and only renders paretic the inhibitory terminations of the vagus. The frog is apparently quite tolerant to atropine. Eight milligrams injected intravenously into a decerebrated frog at one time suspends external respiration promptly (as does 1 mgm. of nicotine). Examination of the lungs shows them contracted. But almost complete recovery sets in within an hour and at this time the lungs assume their normal size and function.

Atropine in this dosage does not paralyze the terminals of the inhibitory fibers of the lung as it does the vagal nerve endings in the heart. In all of these atropinized preparations stimulation of the vagus nerve was without effect on the heart.

Adrenalin. In the frog the irrigation of the lung itself or injection of adrenalin chloride into the circulation causes inhibition of the automatic quick rhythm of the lungs which appears spontaneously as noted in a previous section of this paper or inhibits the hypertonic activity of the lung following section of the vagus. As illustrations of this effect of adrenalin we offer figure 19, *A* and *B*. The former tracing shows inhibition of the quick rhythm when adrenalin was applied to the lung directly at *X*; the latter, inhibition of the hypertonic state of the lung following vagotomy.

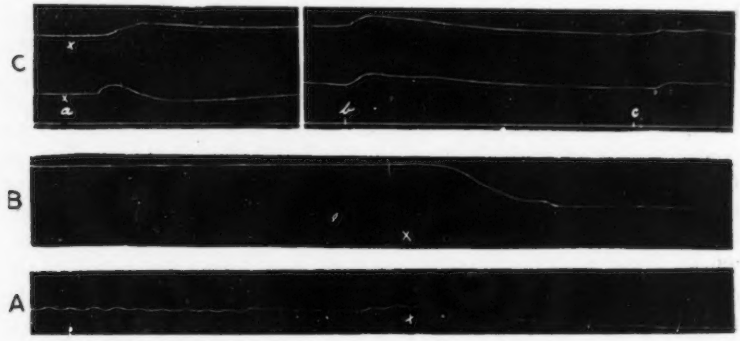

Fig. 19. Water manometer tracings of the intrapulmonic pressure in the frog (R. pipiens). Spinal cord cut and destroyed below medulla. Cannula in tip of lungs. Vagi nerves cut, and lungs isolated from influence of skeletal muscle contraction.

A: *X*, application of $\frac{1}{10}$ cc. adrenalin chloride (1–1000) in Ringer's solution to surface of lung.

B: *X*, injection of $\frac{1}{10}$ cc. adrenalin chloride in 2 cc. Ringer's solution into the heart.

C: Upper tracing, left lung; lower tracing, right lung. Intravenous injections of histamine in 2 cc. Ringer's solution; *a*, 0.01 cc.; *b*, 0.02 cc.; *c*, 0.06 cc. 1–1000 histamine hydrochloride.

Showing inhibition of lung tonus by epinephrin and stimulation by histamine.

Histamine. Figure 19, *C*, and figure 17, *C* at *b* show the effect of histamine-HCl on the neuro-muscular apparatus of the lung when injected intravenously in varying concentrations. In moderately small or large doses it causes invariably a slight contraction of the lung which in amplitude bears no relationship to the dosage. Our experience, as a matter of fact, leads us to believe that successive doses of the drug given within a relatively short period of time have less and less effect because, possibly, of the cumulative poisonous property of this drug.

SUMMARY

1. The actual respiratory movements (opening of glottis and swallowing of air) are accompanied by relaxation of the tonus of the lung musculature, due either to greater action of the inhibitory fibers in the vagi or central inhibition of the motor nerve mechanism, on the assumption that the latter is in tonic activity. This inhibition occurs during the respiratory movements even when no air can enter or leave the lungs. It is therefore of central origin, an effect coördinated with the true respiratory act. From the point of view of utility the inhibition may be designated as a "receptive relaxation." The buccal movements that go on during the period between actual air swallowing do not seem to influence the lung tonus.

2. At the end of the respiratory act there is an active contraction of the lung musculature, after a latent period of 5 to 6 seconds. This contraction is of variable duration (10 to 20 seconds) depending on the respiratory rate and the condition of the lungs. The contraction is usually followed by a gradual tonus relaxation up to the next respiratory act. Occasionally this gradual tonus relaxation is absent. These active lung contractions are best seen during the pause of the Cheyne-Stokes type of breathing, which appears to be normal for the frog. The contraction is cut short by the next swallowing act, so that when the animal is breathing rapidly, the active lung contractions are not in evidence, the lung musculature being in a continuous state of "receptive relaxation." The active lung contractions following a respiratory act can be accounted for by a lowering of the inhibitory influence, thus permitting the peripheral automatism to come into greater play. We have not been able to determine whether motor innervation via vagi and sympathetic nerves also play a rôle as contractions follow the respiratory act even when no air enters or leaves the lungs. It is not a reflex initiated by the stimulation of pulmonary sensory fibers through lung distention. It is probably entirely central in origin and referable to the respiratory center, the inspiratory discharge of this center having the immediate effect of a temporary stimulation of the inhibitory mechanism for the lung tonus, the lung contraction of the end of the inspiration merely signifying excess back swing of the central inhibitory control on its return to the more or less constant level.

3. Section of the cervical sympathetic fibers has no effect on the lung tonus but stimulation of these fibers before they join the vagi

nerves causes lung contractions. The cervical sympathetic nerves contain a few motor fibers, but no inhibitory fibers to the lungs. These motor fibers in the sympathetic do not appear to be in tonic activity, but our experiments on this point are not final.

4. Section of the vagi nerves or destruction of the medulla causes a permanent hypertonus or incomplete tetanus of the lungs. This is due to removal of a tonic inhibitory check on the peripheral lung motor mechanism (peripheral neuro-muscular automatism). Ligation of the pulmonary branches of the vagi produces the same effect. The only part of the central nervous system necessary for this tonic inhibitory control is the medulla. Section and destruction of the entire spinal cord below the medulla has no permanent effect on the tonus mechanism. Decerebration has likewise no permanent effect on it. Destruction of the midbrain causes a temporary diminution of the lung inhibition probably through a "shock" state of the medulla. The afferent aspect of this tonic lung inhibition requires further study. The most important afferent pathway is probably the pulmonary branches of the vagi.

The contractions of the lungs following vagi section are powerful enough to develop a pressure of from 20 to 40 mm. Hg., and if the glottis is not artificially closed all the air in the lung cavity is forced out, the lungs contract down to a solid mass and are thus rendered useless as organs of respiration. All our data on this point are those of acute experiments lasting only 2 to 3 hours. The possible readjustment of this peripheral lung automatism to meet the needs of the animal after double vagotomy is being investigated by long time experiments.

5. Stimulation of the peripheral end of the vagi inhibits the lung tonus induced by vagi section. Strong tetanizing currents applied to the peripheral vagus trunk usually cause strong contractions following the primary inhibition. Nicotine paralyzes the lung inhibitory fibers of the vagi apparently without injury to the motor fibers, so that after nicotine vagus stimulation causes lung contractions only. These contractions are stronger than can be caused by stimulation of the cervical sympathetic nerve. Hence, on the basis of the usual interpretation of nervous action, the vagi carry both inhibitory and motor fibers to the lungs, the former predominating and being tonically active like the cardio-inhibitory mechanism in many animals.

By stimulation of the peripheral end of the vagus we have so far failed to cause a greater tonus relaxation in the lungs than that which

existed before vagi section. This means either that the tonic vagi inhibitory action is ordinarily maximal, or that the simultaneous stimulation of the motor fibers in the vagi trunks neutralizes a part of the inhibitory effects.

The efferent actions of the vagi and the cervical sympathetic nerves on the lungs are unilateral. Only occasionally have we seen effects on the lung of the opposite, in case of sympathetic stimulation. This was probably due to escape of current, and not to actual nerve crossing.

6. Reflex contraction of the lungs are induced by the stimulation of the afferent fibers in the vagi, by stimulation of the cutaneous nerves, the sensory fibers in the nares and the cornea, and the sensory fibers in the visceral organs. These lung reflexes could be brought about either by augmented action of the motor nerve mechanism or by depression of the tonic inhibitory mechanism. On the basis of the usual conceptions of reciprocal innervation both factors are probably involved. It is thus clear that practically all afferent nerves have reflex connection with the medullary nuclei controlling the lung tonus and contractions.

7. As stated above, it is possible to differentiate between the motor and inhibitory fibers in the vagus trunk by means of nicotine. This drug in *moderate doses* (2 mgm.) paralyzes not only the respiratory center but also the peripheral inhibitory mechanism so that subsequent stimulation of the vagus causes more or less powerful lung contractions in place of the usual inhibition resulting from stimulation of this nerve before nicotinization. If nicotine is injected in a frog that has suffered bilateral vagotomy with the usual escape of the tonic inhibitory control of the corresponding lung, nicotine effects a marked inhibition of this lung and after a slight interval an escape from central control of the other lung. The escape of the one lung does not occur until the primary and temporary inhibition (stimulation) of the peripheral mechanism in the other is about over. This probably means that the lung still connected with the center before nicotinization is under maximal central inhibitory control since the drug in this instance produces no primary inhibition. Injection of nicotine in any case destroys the central tonic inhibitory control of the lungs similar to destruction of the medulla or double vagotomy. In large doses nicotine causes contraction of the lung musculature probably by direct stimulation of the muscular elements.

8. Atropine in doses large enough to paralyze the cardio-inhibitory fibers of the vagus has no effect on the terminations of inhibitory fibers in the lungs. In fact, even huge intravenous doses (8 mgm.) do not

completely paralyze these terminations. Such doses paralyze chiefly the medullary centers which send out the inhibitory impulses. As a result the lungs contract. But even this center recovers within an hour and again assumes its tonic inhibitory control over the lungs.

9. Histamine in small or large doses (0.01 to 0.07 cc. 1:1000 sol.) causes temporary contraction of the lung musculature.

10. Epinephrin inhibits both the peripheral automatic tonus and the peripheral automatic rhythm if one is present.

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THE EFFECT OF ADRENALIN ON VENOUS BLOOD PRESSURE

HELENE CONNET

From the Physiological Laboratory of the Johns Hopkins University

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A REVIEW OF THE LITERATURE ON VENOUS BLOOD PRESSURE

Regulation of venous pressure by: 1. Peripheral resistance. It is usually stated that, in general, increased peripheral resistance (in the arterioles)—other things being equal—causes a rise in arterial pressure and a fall in venous pressure and that decreased resistance has the opposite effect.

Bayliss and Starling (7) and Plumier (61) advance the idea that an increased peripheral resistance caused by vasoconstriction decreases the capacity of the vascular system and tends to cause a rise of pressure in all parts of the system. What change will occur in the venous pressure will depend upon whether the influence of the decreased flow from arteries to veins causing a fall, or the decreased capacity of the system causing a rise, predominates. The opposite state of affairs holds in case of decreased peripheral resistance. Sometimes the tendency for the venous pressure to rise is exactly counter-balanced by the tendency to fall. Bayliss and Starling (7) cite, as an illustration, the absence of venous pressure change when vasomotor paralysis has been induced by section of the cord just above the first thoracic segment. Plumier (61) also illustrates this point. He finds no change in venous pressure after weak or strong stimulation of the central stump of the vagus, both vagi being sectioned. In the case of weak stimulation, vasodilatation was produced while strong stimulation produced vasoconstriction. In each case, however, the influence of the peripheral resistance on the venous pressure was balanced by the opposite influence of the change in the capacity of the system.

According to Bayliss and Starling (7), sometimes the venous pressure-raising factor in vasoconstriction of the arterioles predominates, as when the splanchnics are directly stimulated or the vasomotor center

is stimulated by asphyxia. The objections, as advanced by Hill and Barnard (37) and Plumier (61), to attributing this rise in venous pressure to decreased capacity of the vascular system will be discussed later.

2. Heart rate. The authors mentioned above (Bayliss and Starling, and Plumier) state that a rise in venous pressure is obtained when the heart slows or stops beating because there is a tendency toward equalization of pressure throughout the system, resulting in a fall in arterial and a rise in venous pressure due to the elasticity of the arteries forcing more blood into the veins. Usually in a slowly beating heart the output per minute decreases and hence the heart does not pump into the aorta per unit of time as much as it did before, thus causing a back pressure in the pulmonary veins, which eventually affects the right heart and causes a rise in the vena cava pressure. This would be true especially when there was an increased resistance to the blood-flow in the arteries, as in vasoconstriction.

Bayliss and Starling (7) consider that most of the rise of venous pressure after peripheral stimulation of the vagus is due to the decreased capacity of the system which comes as a result of the anemic stimulation of the vasomotor center following the low arterial pressure brought about by such stimulation. Their proof of this is that only a slight rise is occasioned by stimulation of the vagus when either the cord or the splanchnics are cut. Plumier (61) has a different explanation for this. He considers the slowing of the heart of primary importance and the vasoconstriction of secondary. He says that cutting the cord or splanchnics causes such a marked vasodilatation (arterial pressure in one of Bayliss and Starling's experiments fell from 120 mm. to 60 mm. Hg.) that tendency toward equalization of pressures cannot show the effect that it would, if the conditions of the vascular system were normal. This point is perhaps brought out more clearly by considering what changes take place in the vascular system under asphyxia, produced by removal of artificial respiration in an animal whose chest has been opened. Bayliss and Starling offer an explanation for this rise in venous pressure, similar to that given in connection with vagus stimulation. Plumier (61) attempts to prove his point that vasoconstriction is of only secondary importance, by comparing the effect of asphyxia before and after section of the vagi. With vagi intact the arterial pressure rises only slightly but the heart beat soon becomes very slow, and coincident with this slowing the venous pressure in the inferior vena cava and the external jugular rises

markedly. As soon as artificial respiration is renewed and the heart beats faster, the venous pressure falls. In the experiment when both vagi are cut, the arterial pressure rises, due to vasoconstriction, but the venous pressure remains unchanged, until (100 seconds after the beginning of asphyxia) the heart becomes paralyzed and consequently slows, and the blood pressure falls. At this time the venous pressure rises. Furthermore, after artificial respiration has been renewed, the arterial pressure rises, due to continued vasoconstriction, and when it is at its maximum the venous pressure has already fallen to normal.

The difficulty here, it seems to me, is in trying to make either heart-slowing or vasoconstriction alone account for the rise in venous pressure. Bayliss and Starling (7) themselves, when discussing the rise of venous pressure after splanchnic stimulation, say (p. 172): "In experiment 5 one of the vagi was intact and the heart was slowed as usually occurs when the splanchnics are stimulated. One might be inclined to ascribe the rise of venous pressure to this slowing of the heart, were it not that in other experiments where both vagi were divided, we still obtained a slight rise on stimulation of the splanchnics." Evidently, then, the slowing of the heart is responsible for the greater part of the venous pressure rise, if not absolutely all. On the other hand, one cannot see how Plumier can be sure that Bayliss and Starling are wrong in ascribing some of their rise in venous pressure, after asphyxia for instance, to the decreased capacity of the system, since no experiment has been performed in which that factor has been ruled out, granting that Bayliss and Starling's attempt to rule it out, not only ruled it out, but introduced a new factor, that of vasodilatation after section of the cord or splanchnics, which vitiated the comparison. It would seen as though this point might be settled by an arrangement, such as that used by Heard and Brooks (31), for keeping the arterial pressure constant under varying experimental conditions. When, after adrenalin or asphyxia with vagi intact, the change in capacity of the system, due to vasoconstriction, was not allowed to be effective, one could then see how much this factor has to do with the rise in venous pressure on slowing of the heart.

3. "Respiratory pump." According to Hill and Barnard (37) none of the above explanations is adequate in accounting for the rise of venous pressure obtained as a result of various experimental procedures. They attribute the rise which occurs on arrest of the heart under peripheral vagal stimulation to the respiratory spasms produced, and on asphyxia to strong abdominal and general muscular move-

ments, for in curarized animals these rises do not occur, at least in the case of asphyxia. Their curarization was sufficient to abolish natural respiration, but did not interfere with the heart or tone of the arterial system. As regards vagal stimulation, they say (p. 348), "we ourselves unfortunately have not been able to maintain arrest of the heart in the curarized animal for long enough time to settle this point." Yet in figure 12, page 342 of this article, they show a rise in venous pressure in the same animal on stimulation of the vagus before and after the administration of chloroform. "In the first instance the escape of the heart is complete, the respirations are greatly intensified and by the powerful expiratory spasms of the abdominal muscles the venous pressure is greatly raised. In the second case the inhibition is complete while the respirations, weakened by the chloroform, remain unaltered during the standstill of the heart." On examination of the second curve one sees the venous pressure beginning to rise as soon as the beat of the heart is stopped, but while it is rising the animal was changed to the feet-up position. The venous pressure continues to rise until the heart once more begins to beat. This rise of venous pressure evidently cannot be ascribed to the activity of the respiratory muscles since no change in respiration occurs during the standstill of the heart, nor, probably, to the feet-up position which was assumed during the rise of venous pressure.

These authors object to any explanation of these phenomena which assumes that a rise in venous pressure can be brought about by sending more blood into the venous system, as occurs on arrest of the heart, or by decreasing the capacity of the vascular system by arterial vasoconstriction. They believe that the vascular system is not filled to distention and that since the veins can hold all the blood of the body without distention, no increase in amount of blood in the veins or decrease in arterial caliber can cause a rise in venous pressure. To prove that the veins can hold all the blood of the body at zero pressure they cite the condition that exists in the animal after death. Then, since it might be thought that after death the tone of the vascular system had passed off, they attempt to prove their point in a living animal. After stopping the heart by vagus stimulation, they alternately placed the dog in the vertical feet-down and horizontal positions until all the blood had passed from the arteries into the veins, and past the venous valves so that no reversal of flow could take place. They say (p. 346), "In this experiment we produced a positive pressure in the veins and no pressure in the arteries. . . . . Since the arteries are emptied

of blood the whole system is not filled to distention . . . . ." Just because the arteries are much more elastic than the veins and can, when the heart stops beating, empty themselves of blood and produce a greater positive pressure than before in the veins, it does not therefore follow, it seems to me, that the vascular system, while the heart was beating, was not filled to distention.

Furthermore, in refutation of Bayliss and Starling's belief that anemia of the brain in vagal arrest causes vasoconstriction, they say (p. 348), "In our experiments when the heart has escaped from arrest, there has not occurred any great rise of arterial pressure which we should expect to indicate vasoconstriction." Why should one expect any great rise of arterial pressure after the heart has begun to beat again? With the resumption of the heart beat and consequent rise of arterial pressure toward normal, the cause of the vasoconstriction, anemia of the brain, is removed.

"Any appreciable increase of vena cava pressure is due either to the reduction of the capacity of the venous system by the action of the respiratory muscles, or to the failure of the heart in maintaining the systolic output" (p. 350). By this, I presume, is meant the inability of a slowly beating heart to expel per minute as much blood as it receives per minute, thus causing a back pressure in the veins. I fail to see how this could cause a rise in venous pressure if their contention is correct, that the venous system is capable of holding all the blood of the body without distention, especially since it occurs almost immediately on slowing of the heart, before the arteries could have emptied the greater part of their blood into the veins. Of course, it may be contended that the cava is only a part of the venous system and it might become distended when a large amount of blood collected in it, but this hardly seems a valid contention in the case of the immediate rise in venous pressure on arrest of the heart.

4. Chemical mechanism. Roy and Brown (64) in experimenting with the frog's web, tongue and mesentery, found that temporary anemia was followed by dilatation of arteries, capillaries and veins. This dilatation they attribute to a "relative diminution in the lymph of certain of the constituents of the blood, or the presence in increased amount of certain of the products of tissue exchange, or both of these combined" (p. 359). This effect, they say, is independent of cerebrospinal vasomotor effects; it may possibly be due to action on peripheral vasomotor ganglia, but they think it is more probably due to direct action on vessel walls. These causes operate in other congestions and

the authors feel that this automatic regulation of the peripheral circulation is of very great importance.

Henderson and Harvey (33), and Henderson (32) develop the idea of a peripheral chemical control of venous pressure "largely through variations in the CO_2 content of the venous blood." CO_2, by relaxing the veins, they believe, removes the resistance to the flow of blood from capillaries to veins, and so causes an increase in venous pressure. They speak of such a relaxation as though it were merely the complete or partial removal of a clip, allowing more blood to flow into a vessel whose caliber remains the same. But if relaxation means more than this; if it means an actual increase in the capacity of a portion of a blood vessel, due to the lessening of vascular tone, it is difficult to see how this relaxation can cause a rise in the pressure in the vessel even though there is an increased amount of blood present.

5. Nervous mechanism. In a study of the regulation of the blood supply of the brain in 1890, Roy and Sherrington (65) found that stimulation of the peripheral stump of the vago-sympathetic nerves in dogs produced sometimes a rise, sometimes a fall of general venous pressure. They think that this probably means that the vago-sympathetic trunk contains vasomotor fibers to the veins. As discussed in the section on heart rate, later observers have attributed this rise which peripheral vagus stimulation gives, to the slowing of the heart which is occasioned by such stimulation. Slowing of the heart due to paralysis from asphyxia with both vagi cut has been shown by Plumier to give a rise in venous pressure comparable to the rise caused by peripheral vagus stimulation. This perhaps does not prove that the vago-sympathetic nerves possess no venomotor fibers, but there seems to be perfectly adequate explanation for the venous pressure change without assuming the existence of such venomotor fibers. That Roy and Sherrington sometimes found that stimulation of the peripheral stump of the vago-sympathetic nerves produced a fall in general venous pressure, may possibly have been due to an escape of current to the central end of the nerve through the fluid medium surounding the tissues. One cannot tell what happened to the heart rate in these cases of a fall in venous pressure for no graphs are given or statement made in regard to it.

Thompson (72) in 1893 observed constriction of the superficial veins of the hind limb of dogs and rabbits on stimulation of the sciatic nerve or the spinal cord. The constriction took place in short sections, the diameter of the vein between the segments remaining unchanged. Bancroft (3) confirmed these observations and extended the work.

He observed the appearance of the veins of the hind limb of a cat (rabbits also were used but were not found nearly as satisfactory) before and after section of various nerves, and thus traced out the vasomotor supply. The cell body of the pre-ganglionic fiber lies in the spinal gray matter, the axis-cylinder emerging in the anterior root of the 1st, 2nd, 3rd or 4th lumbar nerve, following the corresponding white ramus into the sympathetic chain, running down it for a certain distance. The cell body of the post-ganglionic fiber lies in the 6th or 7th lumbar sympathetic ganglion, the axis-cylinder running to the veins in the sciatic nerve. Langley's nicotine method was used to determine the position of these ganglia.

The experiments of Gunn and Chavasse (30) and Crawford and Twombly (18) on the effect of epinephrin on isolated veins lend support to the belief that a venopressor nervous mechanism exists in the veins. The details of these experiments will be given later in this paper under the head of adrenalin.

Hooker (41), (42) has demonstrated veno-pressor fibers in a nerve trunk running from the inferior mesenteric ganglion to the veins of the large intestine. A rise in venous pressure in an isolated loop of intestine was induced by stimulation of: *a*, the nerve to the part (peripheral mechanism); *b*, a sensory nerve such as the saphenous (central reflex mechanism); *c*, the central mechanism by asphyxia. Section of the peripheral nerve or destruction of the medulla destroyed the reflex. A probable failure of this mechanism in shock is predicated by Morison and Hooker (55).

A contraction of the veins seems to be the cause of the increased capillary pressure found in the blue-handed type of irritable heart cases as described by Briscoe (9). This is especially illustrated in those patients whose hands were sometimes normal and sometimes blue. The average readings of this class, when the hands were normal, were: venous pressure, 10.6 cm. H_2O; capillary pressure 25.3 cm. H_2O; and when blue were: venous pressure, 10.6 cm. H_2O; capillary pressure, 33.3 cm. H_2O. Whether this contraction of the veins falls under the chemical mechanism theory or the nervous mechanism theory, the author does not state. One presumes, from the article, that it is the latter.

It is not intended in this paper to review in detail the literature on portal venous pressure, as it forms a rather specialized type of venous pressure. A comprehensive review of the literature which has established the presence of a vasomotor mechanism in the radicles of the

portal vein, is to be found in Burton-Opitz's (13) and Edmunds' (22) papers.

Effect on venous pressure of: 1. Adrenalin. a. Effect on isolated veins. Dunn and Chavasse (30) have tested the effect of adrenalin (1 to 100,000) on isolated ring preparations from various veins in the sheep (external jugular, mesenteric, superior and inferior cava) and find a constriction similar to that which occurs in the arteries. The external jugular gave a greater response than the superior and inferior cavae. The amount of response of the mesenteric vein could not be compared with that of the other veins, for the temperature in the case of the mesenteric was 41°C., while in the other experiments it was 36°C. From this they judge that the veins probably contain veno-constrictor nerve fibers from the thoracico-lumbar sympathetic nervous system. Crawford and Twombly (18) corroborate these observations, finding constriction of ring preparations from femoral, iliac and saphenous veins of the dog. Their work on roosters is interesting in that they find some veins that contract in adrenalin solutions, and others that do not. Rings of the jugular vein of white Leghorn roosters, taken from the middle of the neck, contract slowly with 1 to 60,000 adrenalin in oxygenated Ringer's solution, but rings from the jugular vein near the head and from the large vein of the wattles gave no response. This argues, they feel, against a vasomotor supply to the cephalic end of the jugular vein and the veins of the wattles, and they suggest that perhaps the absence of vasoconstrictor fibers in wattles may be one of the reasons why they blue so easily.

b. Effect of intravenous injections. Hill (36) found that intravenous injection of suprarenal extract into a dog whose vagi had been divided caused a rise in arterial pressure of 1170 mm. $MgSO_4$ solution, while the vena cava pressure remained unaltered. Plumier (61) attributed the rise in superior and inferior vena cava pressure, which he obtained on intravenous injection of adrenalin in the intact animal (dog), to the slowing of the heart which such an injection occasions. He feels that one does not get as great a rise with say a 0.4 mgm. injection as the slowing of the heart would seem to indicate, but this may be explained by the fact that the increased force of the heart beat tends to reduce the venous pressure. However, after cutting the vagi, unless a very large dose is administered, there is no change or only a slight rise in venous pressure. In both cases there was important vasoconstriction resulting in a considerable rise in arterial pressure, but this decreasing of the capacity of the vascular system, he points out, is not sufficient,

in itself, to change the venous pressure. Capps and Matthews (16) find that a small dose (they do not state the amount) of adrenalin does not affect the venous pressure, but a large dose, such as $\frac{1}{5}$ mgm. (2 minims) of 1 to 1000, causes a rise of from 10 to 80 mm., and that coincident with this rise, the heart is markedly slowed. "The rise in venous pressure was coincident with this halting, irregular action of the heart, and it remained high until the normal rhythm returned. We found likewise that, in a slow or inhibited heart-action from exciting the vagus nerve with faradic current, the venous pressure rose. Hence it seems probable, as Plumier (61) states, that the rise in venous pressure after large doses of epinephrin is explained by the halting heart-action rather than by any venomotor stimulation" (p. 390). Bainbridge and Trevan (2) exclude reflex vagus inhibition in their experiments by a small dose of atropin early in each experiment. Under these conditions they find little or no change in vena cava pressure after adrenalin injection into a portal tributary or systemic vein (hepatic artery tied or intact), but a rise in portal pressure due, they think, either to a swelling of the columns of the liver cells narrowing the capillary channels, or constriction of the radicles of the portal vein. Two cubic centimeters of a 1 to 10,000 solution of adrenalin cause a rise in portal pressure equal to 255 mm. of sodium citrate solution. Kuno (50) explains the slight rise in venous pressure which he obtained on injection of adrenalin, with the heart beating faster, by saying, "the contraction of the blood vessels evoked by adrenaline is most distinct in the arterial system so that a large amount of blood might escape into the veins. The pressure in the veins does not therefore fall, on the contrary it rises more or less during action of the adrenaline although the heart works extremely energetically" (p. 232).

As far as I know, no measurements have been made of the effect of adrenalin on venous pressure in man. A word of caution should, therefore, be given about transferring data concerning adrenalin from animals to man. As has been noted above, the rise of venous pressure in dogs has been attributed to the concomitant slowing of the heart. In man, however, adrenalin does not slow, but quickens the heart rate.

Clinical findings reported by Donaldson (21) and Miller (54) indicate that in practically every person (normal or pathological) adrenalin injection causes no change or causes an increase in pulse rate. Only one case of a fall in pulse rate was reported (Donaldson) and this was in a patient much collapsed from hemorrhage. With the subcutaneous

injecion of 0.5 cc. of 1 to 1000 adrenalin, used in the "Goetsch" test (29), (57), there is no reaction on the part of normal patients and an increase of ten to fifty beats per minute in patients hypersensitive to adrenalin. This result is further corroborated by the work of Wearn and Sturgis (74) and of Tompkins, Sturgis and Wearn (73). Therefore it would be incorrect to assume that the results obtained by Capps and Matthews (16) on dogs, in their studies on "venous blood-pressure as influenced by the drugs employed in cardiovascular therapy," necessarily apply to man. Similarly, Meek and Eyster's conclusion (53) (based on experiments on unanesthetized dogs with good vagal tone), that adrenalin cannot be the immediate cause of cardiac acceleration which follows moderate exercise, because the heart slows after adrenalin, cannot be held good for man in whom the heart increases its per-minute rate after adrenalin. Still another example of the different way in which adrenalin affects the same structure in different animals is given by Barbour (5), who found that adrenalin caused constriction of human coronary arteries, but relaxation of the coronary arteries of the calf, sheep and pig.

2. Various other influences. Since the experimental work in this paper deals only with the effect of adrenalin on venous blood pressure, it does not seem appropriate to review, in detail, the effect of various other in uences on venous pressure. The following references may, however, serve to make this general review of the subject more complete. The relation of venous pressure to:

a. Gravity. Hill (35); Hill and Barnard (37); Barach and Marks (4)

b. Respiration. Jacobson (46); Wertheimer (75); Hill and Barnard (37); Burton-Opitz (12); Plumier (61).

c. Exercise. Burton-Opitz (11); Hooker (38); Elpers (23); Jones (47); Henderson and Harvey (33). Krogh's (49) recent work on the effect of exercise in opening up new capillary beds, is of interest in this connection.

d. High altitudes. Schneider and co-workers (66), (67), (68), (69); Kellaway's (48) work on the relation of anoxemia to the output of adrenalin may throw some light on the question of the effect of high altitudes on venous pressure.

e. Increased atmospheric pressure. Hill (36).

f. Drugs, other than adrenalin. Hill and Barnard (37); Plumier (61); Burton-Opitz (14); Capps and Matthews (16).

g. Injection of physiological solution. Bainbridge (1); Kuno (50).

Venous pressure work on man. A review of various methods for measuring venous pressure in man is found in an article by Hooker and Eyster (43), in the Johns Hopkins Hospital Bulletin for 1908. The following is a list of articles dealing with these methods:—Oliver, 1898, (58); Frey, 1899, (26); 1902, (27); Gaertner, 1903, (28); von Basch, 1904, (6); Sewall, 1905, (70); von Recklinghausen, 1906, (62); Oliver, 1907, (59); Moritz and von Tabora, 1910, (56); Frank and Reh, 1912, (25); A. A. Howell, 1912, (45); Lombard, 1912 (51); Hooker and Reese, 1914, (44); Hooker, 1914, (39); Brown, 1918, (10); Wiggers, 1918, (76). For comparison with the normal venous pressure values in man, given in the above articles, it may be of interest to note the venous pressure values obtained by Jacobson, 1867, (46), in sheep, and by Burton-Opitz, 1903, (12), in dogs.

Pathological values are given in the articles by Calvert (15); Hooker and Eyster (43); A. A. Howell (45) and Clark (17). Diurnal variations are given by Oliver (60); Hooker (39) and Clark (17); the effect of age and sex on venous pressure by Elpers (23) and Hooker (39), (40), and the effect of temperature by Elpers (23); Hewlett (34) and Hooker (39).

AN EXPERIMENTAL STUDY OF THE EFFECT OF ADRENALIN ON VENOUS BLOOD PRESSURE

Introduction. It is evident from the foregoing discussion that in studying the effect of any drug on venous blood pressure, one must take into consideration the effect of this drug on the various mechanisms, changes in which are known to cause changes in venous pressure. So close is the relationship between the various parts of the circulatory, vasomotor and respiratory systems, that any change in one part usually causes changes in several other parts. Hence, a change in venous pressure must very often be ascribed to the operation of several factors, sometimes supplementing, sometimes antagonizing one another. Sometimes one factor is so predominant than any other is lost sight of. This seems to have been the case in the previous study of the effect of adrenalin on venous pressure. A greatly slowed heart causes a rise in venous pressure. Adrenalin, injected into the blood stream of dogs with good vagal tone, causes slowing of the heart and a rise in venous pressure. Naturally, the conclusion was that the rise in venous pressure was due to the slowing of the heart rate, especially since little change in pressure occurred on administration of adrenalin to these dogs after vagotomy.

It has been demonstrated for sheep by Gunn and Chavasse (30), and for dogs and roosters by Crawford and Twombly (18), that adrenalin chloride causes contraction of isolated vein rings. The work of Bainbridge and Trevan (2) indicates that the radicles of the portal vein in the intact animal contract under the influence of adrenalin. Nerves to the veins of the limb have been demonstrated by Thompson (72) and Bancroft (3), and to the portal vein by Mall (52) and others. The experiments of Hooker (41), (42), demonstrate the existence, in the intestinal veins at least, of a nervous constrictor mechanism, which can be stimulated directly or indirectly. In view of this evidence, one naturally wonders whether or not such a nervous mechanism is functioning when adrenalin chloride is introduced into the circulation, and if the general venous pressure rise occasioned thereby is not, in part at least, due to a constriction of the veins. No evidence, as far as I know, has been found that points to such a mechanism coming into play after adrenalin, except in the case of the portal vein.

It is with these things in mind that the present investigation has been undertaken, to ascertain what factor or factors are responsible for the general rise of venous blood pressure which follows the intravenous injection of a solution of adrenalin chloride.

Experimental procedures. In the course of these experiments about fifty dogs and twenty-five cats were employed, weighing, on the average, 8 kgm. and 2.3 kgm., respectively. The experiments on dogs were carried out under ether anesthesia. The cats were decerebrated according to the method described by Sherrington in his recent laboratory manual (71), an anesthetic, ether, being used only during the procedures prior to the decerebration. The decerebrate cat preparations were always kept on an electric pad throughout the experiment.

Arterial pressure was recorded by a mercury or a Hürthle manometer, sometimes by both. Venous pressure was recorded as follows: brass cannulae (7 cm. long by 1 to 1.5 mm. bore, for cats, and 16 cm. by 2 to 2.5 mm., for dogs), connected with manometers containing 2 per cent sodium citrate, were inserted in the external jugular and femoral veins and pushed in past the valves so that the pressures recorded were those in the superior and inferior cavae. Whenever there was any doubt that the cannulae were not properly inserted, a dissection was made at the end of the experiment. The entrance of the superior cava into the heart was taken as the zero level for pressure. To make sure, from time to time, that no clots had formed, the pressure was raised in the manometer, by means of a pressure bottle, to

several centimeters above the pressure existing in the vein and the citrate solution then allowed to run into the vein. This it did promptly, if there was no obstruction. It was always noted on the tracing when such a procedure occurred. Controls were made which showed that injections of such amounts of 2 per cent sodium citrate ($\frac{1}{2}$ to 1 cc.) had no visible effect on the circulatory system, and the amount of fluid injected was insufficient to cause any change in venous pressure.

Simultaneous tracings of arterial pressure, respiration and time relations were made on a smoked paper kymograph. Since the experimenter worked alone, it was necessary to resort to some method of recording, so that venous pressure readings could be made and injections carried out and recorded by one person, and the exact correspondence between the various pressures be noted on the tracing. One lever recorded respiration, another the arterial blood pressure, and a signal magnet, which marked the base line for the arterial pressure (mercury manometer), recorded the duration of the injection and was also connected with a Harvard clock recording half-minutes. In this circuit there was an electric buzzer which signaled every time the marker was recording a half-minute, and at the sound the level of the venous pressure manometers was noted, the pressures later being marked at the proper place on the tracing. On the same line with this marker, and writing only a millimeter or two behind it, a Jacquet chronograph recorded seconds.

Parke, Davis & Company's adrenalin chloride 1 to 1000 solution was used throughout. Before injection, it was diluted with 0.7 per cent sodium chloride to the desired strength, usually 1 to 10,000.

Special experimental procedures, not followed in all experiments, are described in connection with the experimental data obtained from those procedures.

Of course, if small rises of venous pressure are to be considered significant, one must make sure that they are not caused by the introduction of fluid, used in the injection. For this reason, very small amounts of adrenalin solution were slowly injected (in most cases 0.5 to 3.0 cc. in 5 seconds or more), and control experiments were several times made to ascertain how large an amount of 0.7 per cent NaCl had to be introduced to cause any change in venous pressure. One-half to 3.0 cc. were without effect, 5.0 cc. caused a rise of 3 or 4 mm., while 20.0 cc.—a larger amount than was ever employed in adrenalin chloride injections—caused a rise of only 10 or 15 mm. Na citrate solution.

Experimental work on: 1. Changes in peripheral resistance and capacity of the vascular system. Under all conditions studied, adrenalin always caused a rise in arterial pressure due to arterial constriction. The rise was higher for the same dose when the heart rate was increased than when it was decreased, but there was always a rise. Such a constriction, other things being equal, would tend to cause a fall in venous pressure due to the obstruction to the flow from arteries to veins but, on the other hand, the decreased capacity of the system brought about by arterial constriction would tend to raise the pressure in all parts of the circulatory system. In some cases of arterial constriction, as shown by Bayliss and Starling (7) and discussed in the historical review in this paper, the two factors may balance and give no change in venous pressure. There is one factor here which seems to have received little notice in this connection, and that is the rate at which plasma may leave the blood stream under varying arterial pressures. How far this may operate toward compensating the "decreased capacity of the system" factor, is not known. At all events, in the case of adrenalin, when all other causes for change in venous pressure are ruled out, as far as possible, these factors of peripheral resistance and capacity of the system seem to balance, for no change in venous pressure occurs. Chart 1 (I and II) illustrates this point. About a quarter of an hour after the decerebrated cat (chart 1, I) was curarized, according to the procedure described under "respiration," 0.15 mgm. (3 cc.) of adrenalin chloride was injected into the right femoral vein causing no change in vena cava pressure, although the arterial pressure was raised from 34 to 167 mm. Hg.

Chart 1 (III and IV) shows an experiment in which the venous pressure fell after adrenalin, a very unusual occurrence. It is probable that, in this case, the increased peripheral resistance so obstructed the flow from arteries to veins that there occurred a fall in venous pressure. The decrease in heart rate would tend to cause a rise in venous pressure, but this was apparently overbalanced by the factor or factors tending to cause a fall. In any case, it seems evident that the usual rise in venous pressure caused by adrenalin is not brought about, directly, by changes taking place in the arteries, though, as will be explained later, a rise in arterial pressure may assist in causing a rise of venous pressure when it occurs in conjunction with a slowing of the heart.

2. Chemical regulation. It is conceivable that adrenalin may act as a chemical stimulant to venous musculature, independent of any effect

CHART NO. 1

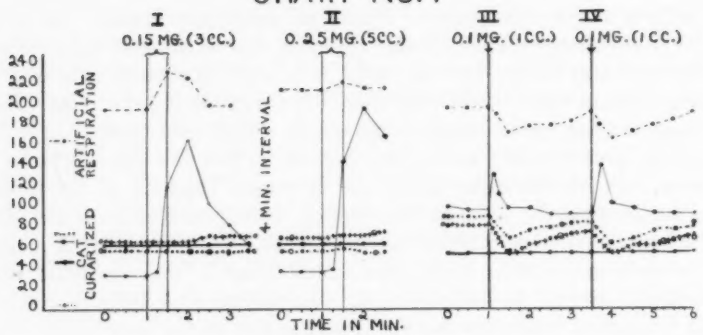


CHART NO. 2

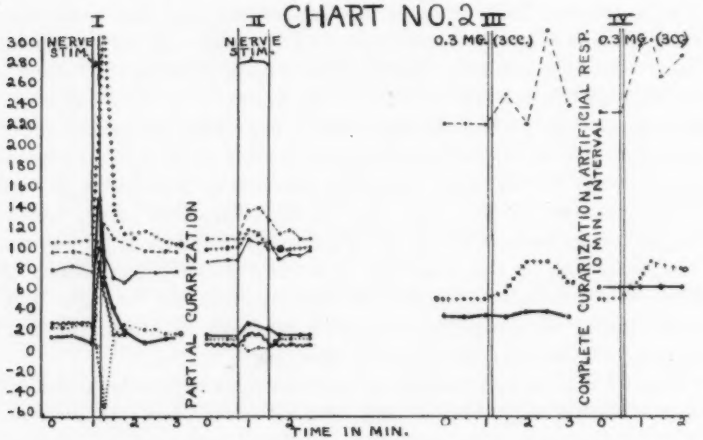
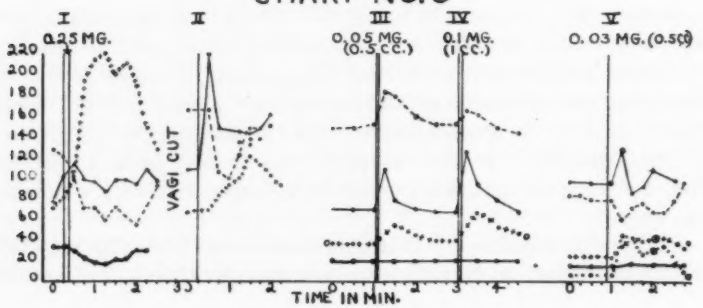


CHART NO. 3



through a nervous mechanism. As far as I know, no work has been done on this subject. However, adrenalin produces an effect upon venous rings similar to that upon arterial rings, and it seems reasonable to assume, until disproved, that in the veins, as has been shown for the arteries, adrenalin acts by stimulating the myoneural junctions of the sympathetic nerve fibers in the vascular musculature.

3. Respiratory and muscular factors. The contraction of muscles against the veins, as in exercise, and changes in respiration, are known to be very efficient in causing changes in venous pressure. Take for example chart 2 (II), showing the result of stimulation of the saphenous nerve before and after administration of curare. The dog was not completely curarized, as can be seen from the fact that a slight change in rate and amplitude of respiration was elicited by strong sensory stimulation. The interesting thing is that the venous pressure response seems to bear a definite relation to the amount of respiratory response. When adrenalin chloride is injected in small amounts (usually 0.1 to 0.3 mgm.) there may be no respiratory response, or a decrease in height and frequency of respiration occurs. This change evidently has little or nothing to do with the rise in venous pressure, since that rise takes place when no respiratory variations occur, and a decrease in rate causes a fall, not a rise in venous pressure. However, to fully rule out any change in venous pressure due to muscular contraction or respiratory change, curare was administered in a number of experiments, and as soon as breathing ceased artificial respiration was given. The air was heated to 30°C. before reaching the animal. To be sure that other muscles besides the respiratory muscles were paralyzed, the

-------- pulse rate per minute.
———— arterial pressure in mm. Hg.
..... superior cava pressure in mm. 2 per cent sodium citrate.
++++ inferior cava pressure in mm. 2 per cent sodium citrate.
▬▬▬ respiratory rate per minute.
Wavy line, respiratory amplitude (relative only).
Large dots indicate actual determinations.
Circles indicate that venous pressure cannula was tested and found free of clots.

Chart 1. Adrenalin chloride injections: I and II, decerebrate cat 7; III and IV, dog 4 B.

Chart 2. I and II, dog 35 A—Stimulation of saphenous nerve before and after partial curarization; III and IV, decerebrate cat 9—Adrenalin chloride injections, before and after complete curarization; vagi cut.

Chart 3. Adrenalin chloride injections: I and II, dog 1 A, vagi cut between I and II; III and IV, dog 4 B; V, dog 29 A.

minimal nerve stimulus necessary to cause contraction of some skeletal muscle in the fore limb was determined before curare, and then sufficient curare was given to cause this stimulus and other stronger stimuli to become ineffective. In such experiments adrenalin had practically the same effect on the venous pressure before and after curare: chart 2 (III and IV) is an example. Changes in the rate of the artificial respiration seem, also, to be ineffective. It has been noticed a number of times that the venous pressure response to adrenalin is not as marked immediately after the administration of curare as it is later. In a few cases, as shown in chart 1 (I and II), the effect persists. This depressant action on venous pressure response is probably due to an action on the nervous mechanism in the veins. That it usually passes off very rapidly, fits in with the general idea that the primary depressant action of curare on the circulatory system is of very short duration.

When not curarized, occasionally the act of injecting was a sufficient sensory stimulus to cause a muscular response. This muscular response, often seen in a stretching out of the lower limbs and consequent stretching of the abdominal muscles, was not evident on the tracing given by the usual pneumograph. Therefore, instead of recording respiration by means of a rubber bag around the chest, a small rubber balloon was inserted through a small opening into the abdominal cavity near the diaphragm, and the skin closed tightly around the glass tube to which the balloon was attached. The balloon was then blown up until it held about 5 cc. air, and connected with a recording tambour and lever as the bag around the chest had been before. This has the advantage of recording not only respiratory changes but also changes in abdominal pressure which very markedly influence venous pressure, as shown by Hill and Barnard (37).

4. Heart rate and output per minute. Plumier (61), in his work on venous pressure, laid great stress on slowing of the heart rate as a very efficient means of raising the venous pressure. Occasionally the heart suddenly stops beating after an injection of adrenalin. The venous pressure may then rise very high; in one case it rose five times as high as it had after a similar dose of adrenalin when the heart rate was increased. In dogs with good vagal tone, the heart usually slows markedly after adrenalin and the venous pressure rises (see chart 3, I). Even with the vagi cut the pulse rate is often slowed, due probably to a direct action on the heart musculature, as is shown in chart 3 (II). A rather uncertain venous pressure response is often seen in vagotomized dogs when the heart rate is increased after an adrenalin injection.

Such results would lead one to attribute the rise in venous pressure solely to the decreased heart rate as did Plumier (61) and, later, Capps and Matthews (16). Chart 3 (III, IV and V), however, makes one doubt that heart rate is the only factor involved. Work on dogs under ether did not prove satisfactory in clearly demonstrating whether or not a nervous mechanism played some part in venous pressure response to adrenalin. There are several reasons for this. In order to rule out the slowed heart rate, the vagi had to be cut. This caused a very great increase in the depth of respiration which brought about such a marked rise and fall in venous pressure that it was difficult to compare such fluctuations in pressure with the change occurring after adrenalin, since the respiratory variations were then often absent, due to temporary cessation of respiration. Besides, even with the vagi cut, the heart frequently slowed after adrenalin. Still more serious, perhaps, is the possible effect of ether on the nervous mechanism. Ether depresses the arterial vasomotor response to adrenalin, as shown by Berry (8) and Rous and Wilson (63), and could naturally be expected to affect a nervous mechanism in the veins—all the more so because, as shown by Hooker (41), this mechanism is extremely sensitive. If very light ether anesthesia were employed, there would then be the difficulty of muscular and respiratory responses discussed above. Chart 3 (III and IV) shows an unusual experiment in which the ether anesthesia was light, and yet respiration remained constant throughout the experiment. Experiments on decerebrated cats, which needed no anesthetic after the operation, and whose vagal tone is not nearly so marked as dogs', proved to be very satisfactory. Also, it was no small consideration, since curare is very difficult to obtain, that it took only a small amount of curare to curarize a cat, as compared with a dog. Since sensory stimuli have been shown to be effective after curare, it is desirable, for humanitarian reasons, to work on a decerebrated preparation where there can be no question of not giving enough anesthetic during curarization to cause analgesia.

Concerning the influence of change of the heart rate on venous pressure, it should be said that it is really the per-minute output of the heart, rather than the heart rate alone, that is essential. Quoting from Erlanger and Hooker (24), (p. 161), "If the pulse pressure is approximately dependent upon the amount of blood that escapes from the arteries during one cardiac cycle, then it is obvious that the amount that escapes must vary directly as the pulse rate." Therefore, pulse pressure multiplied by pulse rate gives us an expression of the output

of the heart per minute. It is possible to increase or decrease the output per minute by increasing or decreasing either or both of these factors, and to keep the output constant by varying the two factors proportionately in the opposite directions. Hence one cannot say that a slowed heart necessarily causes a decreased output per minute, and hence a rise in venous pressure. It is theoretically possible that the pulse pressure might so increase that even with a lower heart rate the output per minute would increase. In the case of adrenalin, however, this possibility seems never to be realized, at least when the pulse rate is greatly reduced. For example, in one experiment, before adrenalin, the pulse pressure was 180 mm. Hg. and the pulse rate per minute 158, making a per-minute output of 28,440. After adrenalin, the pulse pressure was 240 and the pulse rate 20, giving the greatly decreased per minute output of 4800. The slowing in this case was way out of proportion to the increased pulse pressure, and was accompanied by a rise in venous pressure from 25 to 120 mm. Na citrate.

Experiments were performed on dogs, as illustrated in chart 4, in which the vagi were cooled by perfusing ice water through glass tubing in a V around each nerve, and afterwards allowing the nerves to come to room temperature again. The pulse rate, arterial pressure, respiratory rate and amplitude were all affected but very little change in venous pressure took place. When the perfusion of ice water around the nerves was discontinued, the pulse rate decreased from 144 to 108, the venous pressure decreasing also a few millimeters. When the heart stops beating the rise in venous pressure is due to a back pressure in the veins and also to the tendency for equalization of pressures to take place throughout the vascular system. When the heart slows, but does not stop, these factors operate to cause a rise in venous pressure, but to a less extent. If now the arterial pressure rises as the slowing of the heart occurs, as after adrenalin, the mean pressure of the vascular system toward which the various pressures are tending will, of course, be greater, and hence there is a much greater possibility of a large rise in venous pressure. In one experiment, after an adrenalin injection, the heart was slowed about as much as after the cooling of the vagi was discontinued (chart 4, II). In the former case the arterial pressure rose, showing that the capacity of the vascular system was decreased, while in the latter it fell. In the case of adrenalin there may be a nervous constrictor mechanism in the veins at work, but from other experiments on dogs it seems likely that this plays little part in etherized dogs. It seems more probable that the

decreased capacity of the system acts in connection with the slowed heart rate to cause a rise in venous pressure.

5. Nervous mechanism. It can readily be seen from the foregoing that one cannot attribute a rise in venous pressure to the activity of a vasomotor mechanism in the veins, unless one is sure that the per-minute output of the heart has not decreased sufficiently to explain the rise. As we have seen, when this factor is ruled out in experiments on dogs under ether, the evidence for the functioning of a nervous mechanism is not very conclusive. In the experiments on decerebrate cats, however, there is clear evidence of such a mechanism. With the vagi intact, we may get the response which is usual in dogs—a rise in venous and arterial pressure and a slowing of the heart, chart 5 (I and II). When, however, the vagi are cut (chart 5, III and IV), there is still a rise in venous pressure. The heart rate is practically unchanged and the pulse pressure increased (as nearly always occurs after adrenalin) so that the output per minute is now increased and the tendency would be for a fall rather than a rise in venous pressure. So also in this experiment, the respirations were decreased in height and frequency which would tend to lower rather than raise venous pressure. The rise, therefore, which one does get under these circumstances seems to be due to a nervous factor, rendered probably less effective in producing a rise because of the factors tending to produce a fall. Chart 6 shows very uniform rises in venous pressure, though in some cases the heart rate is slowed and in some cases increased in frequency. In the experiment shown in chart 7, the respiratory factor is completely controlled by curarization. The vagi are cut in IV and V.

It is interesting to inquire whether or not the seat of this action of adrenalin is central or peripheral. Two different types of experiments were performed to investigate this question. In the first, the cord was sectioned in the cervical region (about at the level of the fifth or sixth cervical vertebra). As is shown in chart 8, this does not destroy the venopressor response to adrenalin, though whether or not any small part of the reaction is of central origin is hard to determine, since the amount of response to a certain dose is not invariable, and so the effect of a dose before cutting the cord is difficult to compare with the same dose just afterwards. The response before and after is quite similar, however.

For the second type of experiment the isolated vein preparation of Hooker (41) was used. In the first of these experiments, Doctor Hooker kindly demonstrated the method to the writer. An isolated loop

CHART NO. 4

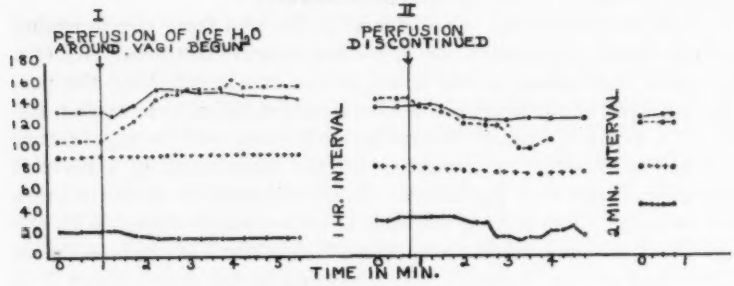


CHART NO. 5

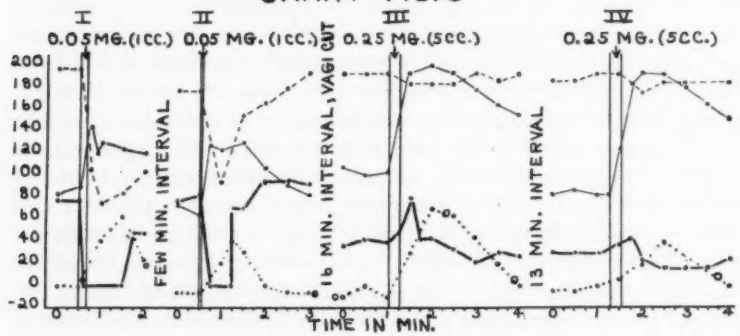
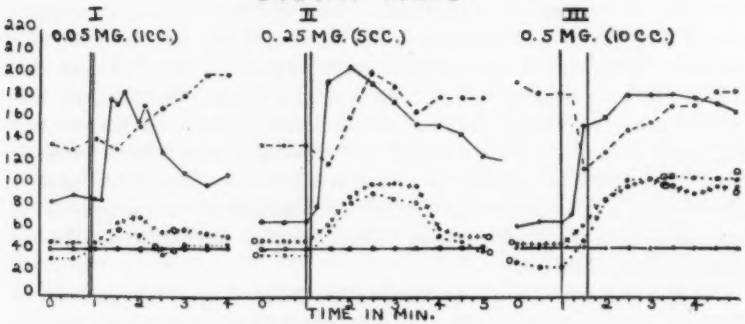


CHART NO. 6



of large intestine in which the inferior mesentery artery and vein had been cannulated, was hung up free from the surrounding intestines. The only connection with the animal was through a nerve running from the inferior mesenteric ganglion to the large intestine and entering the intestine in close proximity to the inferior mesenteric artery. Blood was washed out of the vessels from artery to vein by means of warmed (about 37°C.) Ringer's solution under air pressure of 90 to 120 mm. Hg. The cannulated artery was then disconnected from the air pressure apparatus and allowed to hang freely from the preparation. The vein was then connected with a venous pressure manometer containing warmed Ringer's solution. The zero level was, in most cases, adjusted to the level of the vein in which the pressure was being measured. The vein was distended by a positive pressure of from 30 to 100 mm. Ringer's solution, by means of a pressure bottle. Any change in the caliber of the vein would then be indicated by a rise or fall in the level in the venous pressure manometer. Hooker (42) has shown that, in this preparation, a rise in venous pressure may be elicited, peripherally, by stimulation of the nerve to the part; reflexly, by sensory stimulation; and centrally, by asphyxia. In such a dog, when adrenalin chloride was injected into the saphenous vein in doses from 0.2 to 0.6 mgm., no rise in venous pressure in the isolated vein occurred, but when such injections were followed by asphyxia, produced by closing off the end of the tracheal cannula for two or six minutes, a rise in venous pressure from 15 to 20 mm. occurred, showing that the preparation was still in good condition. This seems to show that the venopressor rise after adrenalin is not due to any central mechanism, controlling the caliber of veins, but to a peripheral effect on sympathetic endings, such as takes place in the arterioles.

------ pulse rate per minute.
——— arterial pressure in mm. Hg.
..... superior cava pressure in mm. 2 per cent sodium citrate.
++++ inferior cava pressure in mm. 2 per cent sodium citrate.
▬▬▬ respiratory rate per minute.

Large dots indicate actual determinations.

Circles indicate that venous pressure cannula was tested and found free from clots.

Chart 4. Dog 17 A—Vagus nerves cooled.

Chart 5. Decerebrate cat 3—Adrenalin chloride injections, vagi cut between II and III.

Chart 6. Decerebrate cat 6—Adrenalin chloride injections, artificial respiration.

CHART NO. 7

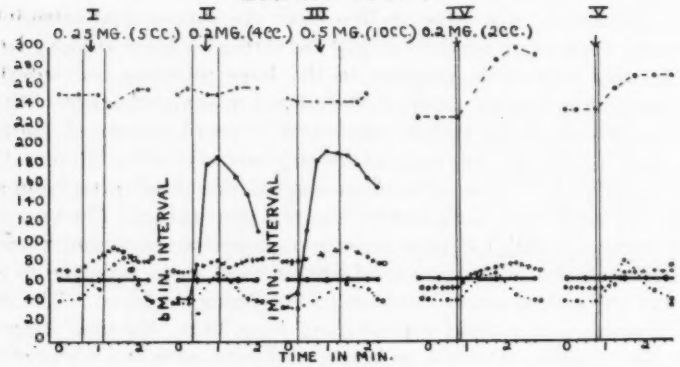


CHART NO. 8

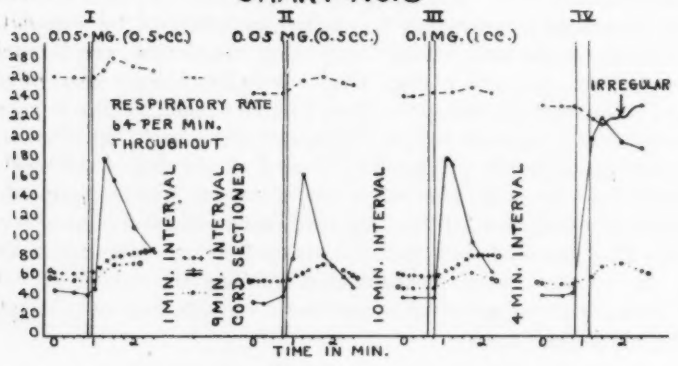
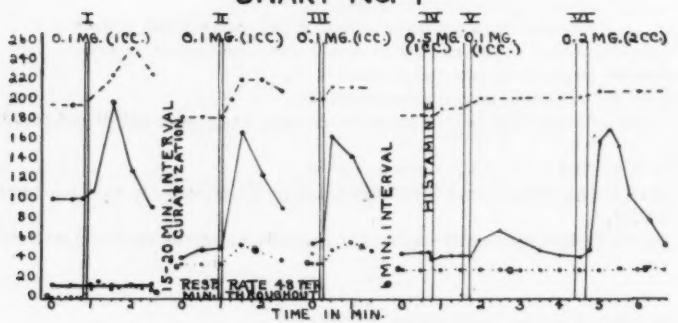


CHART NO. 9



Some experiments which were being carried out in this laboratory suggested that histamine might have an effect on the venopressor mechanism. Chart 9 (I, II, III) shows typical arterial and venous responses to adrenalin in a curarized, decerebrate cat. One-half milligram (1 cc.) of histamine (chart 9, IV) caused a fall in arterial pressure; in some experiments the venous pressure also fell. A more marked reaction was shown in another experiment, where twice the dose was given. According to Dale and Laidlaw (19), had the cat been under an anesthetic, these doses of histamine, from which the unanesthetized cat recovers, would have produced fatal circulatory collapse. When histamine was followed by adrenalin in the same and larger doses than given previously in the experiment, there was no venous pressure response (chart 9, V and VI). The arterial pressure rose, but not nearly as high as before, with the same dose. A second injection, however, gave a typical arterial pressure rise, but still no change in venous pressure. The work of Dale and Richards (20) and Dale and Laidlaw (19) leads them to conclude that relaxation of the capillaries is the cause of the vasodilator effect of histamine. The fall in venous pressure after histamine and the subsequent failure of the nervous mechanism to respond to adrenalin, indicates that histamine has a relaxing effect on venous tone, also, and acts in some way to depress the activity of the venopressor mechanism.

Summary. The various possible factors operating to produce the rise in venous blood pressure which occurs in dogs and cats after intravenous injection of adrenalin are discussed. The two factors chiefly responsible are the decreased heart rate bringing about decreased unit output of the heart, and a vasoconstrictor mechanism in the veins. The

------ pulse rate per minute.
———— arterial pressure in mm. Hg.
..... superior cava pressure in mm. 2 per cent sodium citrate.
++++ inferior cava pressure in mm. 2 per cent sodium citrate.
▬▬▬ respiratory rate per minute.
Large dots indicate actual determinations.
Circles indicate that venous pressure cannula was tested and found free from clots.

Chart 7. Adrenalin chloride injections: I, II and III, decerebrate cat 8—Vagi intact; cat curarized; artificial respiration; IV and V, decerebrate cat 9—Vagi cut; cat curarized; artificial respiration.

Chart 8. Decerebrate cat 15—Adrenalin chloride injections, before and after section of cord; vagi cut; cat curarized; artificial respiration.

Chart 9. Decerebrate cat 17—Adrenalin chloride injections, before and after histamine; vagi cut; cat curarized and artificial respiration begun between I and II.

effect of the first factor is accentuated by the fact that the arterial pressure is greatly raised by adrenalin in the doses here used. The first factor has been recognized before. Reasons are given why the second factor was previously overlooked. In dogs with good vagal tone and under an anesthetic, the rise in venous pressure is almost entirely due to the first factor. In cats whose vagal tone is not nearly as strong, the second factor predominates. This nervous mechanism is shown to be acted on peripherally by adrenalin, and to be depressed by ether, curare and histamine, especially the last.

I may take the opportunity here to thank Dr. W. H. Howell and Dr. D. R. Hooker of this University, and Dr. J. L. King of Goucher College, for kindly encouragement and aid in the preparation of this paper.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

II. Lung Automatism and Lung Reflexes in the Salamanders (Necturus, Axolotl)

A. B. LUCKHARDT and A. J. CARLSON

From the Hull Physiological Laboratory of the University of Chicago

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In a previous article (5) we showed that the lung of the frog contracts immediately following the external respiratory act; that the lung musculature can be induced to contract reflexly by the stimulation of any visceral or cutaneous nerve with practically no exceptions; that the lungs of this animal possess a peripheral automatic mechanism which at times shows rhythm but which under normal conditions is kept in a state of inhibition by tonic central inhibitory impulses via the vagi; that these nerves, furthermore, contain not a few motor fibers which apparently exert no marked tonic activity; and that the cervical sympathetic nerves contain but few motor and no inhibitory fibers for the lungs. In addition, we made a brief study of the action of certain drugs (atropine, adrenalin and histamine) on the lungs besides using nicotine extensively in differentiating between the motor and inhibitory nerves contained in the vagus fibers to the lungs.

On completion of this work it seemed quite important to us to investigate the neuro-muscular apparatus and physiology of the nerves of the lungs of other available amphibia to determine whether or not the physiological mechanism discovered in frogs is the same or similar in this class of animals.

METHODS

The only amphibia available for this study were the axolotl and necturus. The general methods of study were those reported in our first article, modified to meet the anatomical peculiarities of the type of animal which we were studying.

In every instance the cord was pithed below the medulla. As in previous work on the frog we cannulated the tips of the lungs exposed

by a ventral incision and connected each cannula with either a water manometer or delicate tambour. After recording the normal respirations or respiratory attempts of the animal, we attempted to elicit reflex lung contractions as the result of mechanical and electrical stimulations applied to the animal anterior to the spinal transection. We subsequently closed the glottis with a small mosquito forceps (in axolotl only) and pithed the brain (specifically the medulla) to note any tonic inhibitory influence the vagi nerves might have on the neuromusculature apparatus of the lungs. Stimulation of the same nerves to the lungs next engaged our attention. This was followed by the effect of the intravenous injection of drugs alone or in combination. Because of the poor or imperfect circulation in necturus, as a result of the preparation of the animal for experimentation, for certain purposes we injected the drug deep muscularly, 15 to 20 minutes, before preparing the animal in the manner described above; for we found that even injection of the drug in Ringer's solution by way of the bulbus arteriosus or into the pulmonary artery failed to reach the posterior end of the lung of this animal.

The lungs of necturus are paired sacs, extending from the level of the heart almost to the anal region. There are no alveoli or septa in these lungs. According to Miller (9) the course of the smooth muscle fibers in the lung walls is circular, except at the apex. Both lung artery and lung vein lie superficial to the muscle layer.

Miller has described medullated and non-medullated nerve fibers and nerve nets in the necturus lung. The pulmonary fibers of the vagi enter the base of the lungs in many branches, not along the pulmonary blood vessels, as in the frog, but between the blood vessels. There are many ganglion cells (bipolar and multipolar) in the main nerve trunks and in the nerve plexuses. According to Miller, the non-medullated fibers connect with the ganglion cells. Similar ganglionated nerve plexuses have been described in the lungs of other tailed amphibians, especially by Stirling (11) in the case of the newt. The older anatomical literature is reviewed in detail by Oppel (10). Miller's description of the ganglionated nerve plexuses in the necturus lung is practically identical with the local nervous system of the necturus heart, as studied by one of us (6).

In necturus the glottis or opening from the pharynx into the trachea is exceedingly small, and certainly not suited for rapid filling or emptying the lung sacs with air. We have repeatedly seen these animals attempt to swallow air, the air escaping by the gill slits and in no instance

entering the lungs. There can be no doubt that in necturus the gaseous exchange is carried out by the gills, supplemented by the skin, and the rôle of the lung sacs in respiration is an open question. The lung sac of necturus has the same histogenesis as the lung of other vertebrates, although it remains most primitive as regards differentiation. Should these structures be called lungs, especially if they serve mainly or exclusively as "hydrostatic organs?"

In our review of the anatomical and zoölogical literature on amphibian respiration, we came across the surprising fact, apparently well known to zoölogists though not to physiologists, that many species of tailed amphibians have neither lungs nor gills, and in other species without gills the lungs appear to be too rudimentary to function in respiration. The normal condition of these species is that of the frog with the lungs extirpated, that is, the gaseous exchange is carried out entirely by the skin. But several zoölogists (Wilder (12), Lönnberg (8), Camerano (4), Bethge (3) and others) have concluded mainly on anatomical grounds (great vascularity of the mucous membrane of the buccal cavity and upper end of the esophagus), that the pharyngeal cavity serves lung functions, especially in those species having neither lungs nor gills. It appears to be a fact that the so-called buccal respiratory movements (including that of the external nares) are carried out even in those species in which the lungs are absent. But Babák and Kühnova (1) have shown that the brain centers controlling these movements are different from those governing the filling and emptying of the lungs, the latter are, the former are not influenced by asphyxia, which fact seems to throw doubt on the respiratory character of these buccal movements. Lapique and Petetin (7) have also shown, in the case of one species of salamanders devoid of lungs and gills, that the main respiratory organ is in the skin.

None of these questions can be settled except by direct physiological experiments, but we may regard the necturus lungs, provisionally at least, as lungs on the basis of organogenesis. Their motor control also places them in the same category with the lung of the frog and of the axolotl.

Since the entire respiratory apparatus (including the lung) of the necturus is strikingly more primitive than that of the other salamander used in this study, we shall describe the results obtained from necturus first and end with a description of those obtained from the axolotl.

Necturus maculatus. External respiration in this animal is essentially performed by the large gills which under usual conditions are kept in

more or less constant motion. Occasionally the animals come to the surface for air which is promptly expelled by an act of swallowing through the gill slits. The lungs of this animal consist essentially of two thin elongated muscular sacs which are well supplied with blood vessels. With but one exception we found them collapsed. In an inflated and atonic condition their diameter is about 1 cm. at their greatest circumference. At their ends they taper off into blunt tips, at their base they communicate with a tracheal sac similar to that possessed by the frog. This sac communicates with the oral cavity through a glottis situated far down in the pharyngeal region. The glottis is exceedingly primitive. It consists essentially of a slit which is quite easily overlooked on direct inspection. We agree with the description of Oppel that the glottis is exceedingly delicate. Taking everything into account we feel that the lungs may serve an excretory function (elimination of CO_2) but are rarely if ever filled with air during any act of external respiration. The lungs receive their innervation through pulmonary fibers carried by the vagi. It was found impracticable to isolate these latter nerves in the neck for direct electrical stimulation. They were isolated at their exit from the skull by a dissection to right and left of the median line after a sagittal section of the skull.

Because of the inaccessibility of the glottis for closure by hemostat as practised in the frog, we prevented communication of the lungs through the glottis with the mouth by dorso-lateral traction and fixation in that position of the fore legs after pinning the animal on its back. A wad of cotton wedged under the neck of the animal at the level of the tracheal sac or put over the tracheal sac and held firmly in that position by the constriction of a rubber band was an additional measure employed in not only closing off the glottis but in preventing intercommunication between the lungs through the tracheal sac.

Axolotl. The lungs of this genus of amphibia are certainly more complex than in necturus. On opening the abdominal cavity one is at first glance struck with the resemblance of the lungs of this animal with the reptilian lung. The upper portions of both lungs are subdivided into alveolar sacs by septa; the lower appendages which extend down the abdominal cavity for a considerable distance are more saclike in their texture. As in the frog and necturus the lungs communicate at their base with the tracheal sac which in turn communicates with the mouth cavity through a well-developed glottis. In the specimens which we used gills were present and functioning. It was equally apparent, especially on opening the abdominal cavity, that the animals

could and did make use of the lungs for gaseous exchange. In this animal the vagi could be isolated in the neck as in the frog. The result of our investigation on this form is confined to a study of less than a dozen animals. Although most of the animals were in good condition at the time of experimentation, they deteriorated more quickly than the frog and the necturus similarly prepared. We are confident, however, that our results are characteristic of this form especially since they fit in well with what we observed in the frog and necturus.

All the tracings reproduced with this report were taken with the same speed of the kymograph. A single time tracing showing 5 second intervals will be found at the bottom of figure 1. It can be used in a study of the time relations in all other tracings should the reader care to do so.

RESULTS

1. Necturus maculatus: a. The central inhibitory control of the lungs through the vagi. As mentioned above it was found impossible for anatomical reasons to isolate the vagus nerve or its pulmonary branches in the neck to note the effect of ligation and sections of these nerves on the tonic activity of the lung musculature. Since destruction of the medullary centers in the frog effected the same result, we adopted this expedient in necturus. Having closed the glottis and prevented intercommunication of the lungs as described above we connected the lungs each with a water manometer and destroyed the brain entirely by rapidly pithing it. Figure 1, *A*, shows the effect of such a procedure. The destruction at *a* of the cerebral lobes and midbrain was followed by a temporary escape of the lungs from inhibitory control. The subsequent destruction at *b* of the medullary centers was followed by permanent hypertonic state of the lung as in the frog. In another animal whose cord was not pithed at all but which lay quietly on its back as a result of a rubber band placed tightly about the front legs,[1] ligation of the base of one lung was followed by an immediate escape of this lung from tonic inhibitory control in a manner identical with destruction of the medulla (fig. 1, *B*).

[1] Both in the frog and in the necturus pressing the front legs tightly together with a rubber band renders the animals quiescent, and they will lie quietly on their backs for long periods without attempting to turn to a normal position. This procedure depresses also some of the skeletal reflexes.

It should be noted that in this animal the entire central nervous system was intact and that there was the minimum trauma produced in exposing the tip and base of one lung. Nevertheless, on severing this lung from its physiological connection with the central nervous system, the typical lung tetanus was produced. The results of this type of experiment strengthen our position that the striking peripheral automatism of the amphibian lung is a normal physiological state and not induced by the trauma rendered necessary by the experimental procedures.

From these experiments it would seem to follow that in this animal the lung is kept in tonic inhibition by central vagal control. If now one proceeds further and stimulates the peripheral end of the exposed vagus nerves at the base of the skull as was done in many animals, one obtains temporary escape of the lung from the hypertonic state as is shown in figure 2. This animal suffered at *a* an exposure of the anterior end of the brain by resection of the end of the upper mandible. This operative procedure was followed possibly by a slight inhibition of the lung tonus. The medulla was rapidly pithed at *b* resulting in permanent hypertonus of the lung. Stimulation of the peripheral end of the left and the right vagus at *c* and *d* respectively was in each instance followed by a temporary inhibition of the lung with an unusually rapid return to its hypertonic state. If the peripheral vagus stimulation is of sufficient strength the inhibition of the lung tonus usually puts the lung back temporarily to the identical tonus state prior to the destruction of the medulla. This is additional evidence that the state of the lung tonus in the intact animal is governed by the inhibitory control through the vagi.

Since ligation of the base of the lung effects the same result as destruction of the medulla, namely, an escape of the lung from its state of inhibitory control, it might be expected that electrical stimulation of the base of the lung would cause an inhibition comparable if not identical with stimulation of the peripheral end of the vagus. Figure 3 records the result of such an experiment. The lungs being in a state of hypertonus as a result of destruction of the medulla by pithing, stimulation of the vagus of the right lung at *a* caused an inhibition which is virtually duplicated by stimulation of the base of the left lung at *b*.

It appears to us from these observations that there is no escape from the conclusion that the vagi nerves possess inhibitory fibers for the lung which under normal conditions exercise a maximum inhibitory control over the lungs by tonic impulses from the medullary centers.

Fig. 1

Fig. 3

Fig. 2

b. Peripheral lung rhythm. In two instances the lungs of necturus showed a type of tonus rhythm seen occasionally in the frog. Figure 4, *A* and *B*, are records taken from these animals. In figure 4, *A*, this tonus rhythm appeared on pithing the medulla at *a*. Here a fast and later a slow tonus rhythm is written on the curve indicating release of the lung from central vagus inhibition. In figure 4, *B*, the tonus rhythm followed a decidedly rapid inhibition resulting from faradization of the base of the lung at *a* with a strong tetanizing current.

The interpretation of these results is based entirely upon speculation. It is possible that the rhythm which appeared as a result of the destruction of the brain (fig. 4, *A*) arose from occasional inhibitory impulses reaching the lungs through the vagi from more or less intact portions of the medulla which escaped complete destruction during the pithing of the latter. The tetanization with a strong current of the base of the lung in figure 4, *B*, at *a* might have effected changes in the physiological state of the automatic tissue present in the lungs which initiated an occasional and recurring refractory state of this peripheral automatic

Fig. 1. Water manometer tracings of the intrapulmonic pressure in necturus. *A:* Spinal cord cut and destroyed below medulla, cannula in tip of lungs. Glottis closed by dorsal traction on front legs. Lungs isolated by ventral median incision; *a*, destruction of cerebral lobes and midbrain; *b*, destruction of the medulla. Showing permanent lung hypertonus on destruction of medulla.

B: Animal rendered quiet by tying rubber band around front legs, placed on dorsal side, no restraint, with water running over gills. Abdominal incision at base and tip of lung; *x*, ligation of base of lung. Showing permanent lung hypertonus identical with that following destruction of medulla. Time tracing: 5 second intervals.

Fig. 2. Water manometer tracings of the intrapulmonic pressure in necturus. Upper tracing, left lung; lower, right lung. Spinal cord cut and destroyed below medulla; cannulae in tips of lungs. Lungs isolated by a ventral median incision. Glottis (trachea) closed by pressure exerted by dorsal traction on front legs.

a, Transverse section of upper mandible exposing anterior end of brain.

b, Pithing brain.

c, Stimulation of left vagus at base of skull.

d, Ditto, right vagus.

Showing hypertonus of lungs induced by destruction of the medulla, and inhibition of this tonus by vago stimulation.

Fig. 3. Water manometer tracings of the intrapulmonic pressure in necturus. Cannulae in tips of lungs. Spinal cord and brain destroyed. Lungs in hypertonus. Upper tracing, left lung; lower, right lung; *a*, stimulation of right vagus at base of skull; *b*, stimulation of base of left lung.

Showing identical inhibitions of the lung tonus by vagus and by direct lung (base) stimulation.

Fig. 4. Water manometer tracings of the intrapulmonic pressure in necturus. Cannula in tip of lung. Spinal cord cut and destroyed below medulla, lungs isolated by ventral median incision.

A: A pithing of the medulla showing a tonus rhythm of the lungs following destruction of the brain.

B: Lung in hypertonus from destruction of the brain; *a*, direct stimulation of the base of the lung with a weak tetanizing current.

Showing primary inhibition of lung tonus followed by a tonus rhythm.

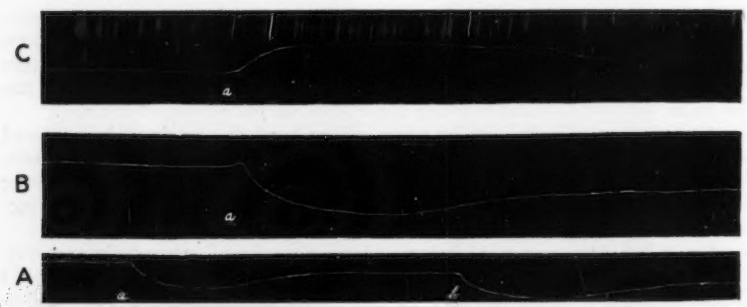

Fig. 5. Water manometer tracings of the intrapulmonic pressure in necturus. Cannula in tip of lung. Brain and spinal cord destroyed, giving the lungs permanent hypertonus.

A: a, injection 0.5 cc. adrenalin; *b*, 0.6 cc. of (1:1000) adrenalin in 5 cc. Ringer's into heart. Showing inhibition of lung tonus by adrenalin.

B: a, injection of 3 mgm. nicotine into heart, showing inhibition of lung tonus by nicotine.

C: a, injection of 0.6 cc. histamine (1–1000) into heart after partial recovery of preparation from a previous injection of nicotine. Showing direct stimulating action of histamine on lungs after paralysis of inhibitory nerve mechanism by nicotine.

mechanism, the inhibition of the hypertonic state of the lung giving rise to the appearance of a rhythm.

c. Reflex lung contractions as a result of cutaneous stimulation. We have been unable to effect reflex contractions of the lungs of the necturus by electrical or mechanical stimulation of cutaneous nerves anterior to the spinal transection.

d. The action of pituitrin, histamine, nicotine and adrenalin on the hypertonus of lung. In most of the work on drugs a cannula was tied into bulbus arteriosus. The drug was diluted with Ringer's and the injections made under moderate pressure through the bulbus. The method was poor even under favorable conditions. Direct intravenous injection was out of the question because active circulation through the lungs was absent in every animal although heart appeared in good condition. Even when the drug was injected under pressure we never felt certain that it reached all parts of the lung. This was due partly to the peculiarity of the pulmonary circulation in this animal and partly to the fact that a good deal of the fluid containing the drug escaped from the vessels ruptured in destroying the brain and isolating the vagi.

Adrenalin. The injection of adrenalin caused a marked inhibition of the hypertonic lung as is recorded in figure 5, tracing *A*, at *a* and *b*.

Pituitrin. According to the commonly accepted view, pituitrin is a direct stimulant of smooth muscle tissue. We were somewhat surprised to find that this drug in small or large doses causes a prompt and marked inhibition. Figure 6, *A*, is a record taken from an animal which suffered destruction of the medulla at *a* followed by a hypertonic state which was markedly reduced by the injection of pituitrin at *b*. The slow recovery is also recorded. No attempt was made to determine the point of action of pituitrin. Pituitrin not only caused a relaxation of the lung; but when weak solutions of this drug were used to irrigate the intestines all intestinal movements ceased after a short latent period. It would seem, therefore, that in these animals pituitrin does not act as a direct muscular stimulant but rather as a stimulant of the inhibitory mechanism.

Histamine. Figure 6, *B*, illustrates at *c* the typical inhibitory effect of this drug in an animal whose lungs were in hypertonus as a result of destruction of the medulla at *b*. Both lungs showed pronounced relaxation with very slow recovery. In all types of animals other than necturus this drug causes a more or less powerful contraction of the lung musculature (turtle, frog). Necturus proved to be an exception

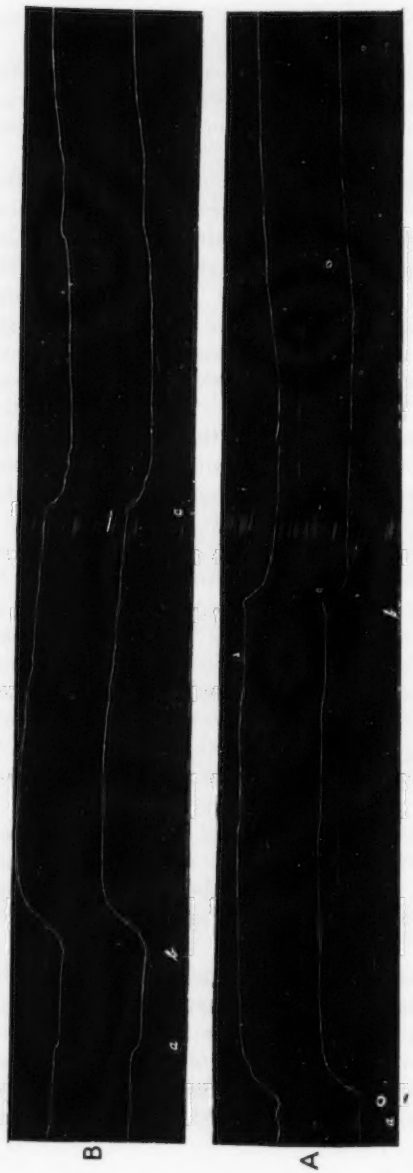

Fig. 6

Fig. 7

to the generally accepted view that the drug supposedly acts as a direct muscular stimulant. The results obtained suggest the possibility that the drug acts primarily on the inhibitory mechanism. We therefore attempted to eliminate the latter by means of nicotine. As a matter of fact we found that the injection of histamine after previous nicotinization effected a contraction of the lung in every instance as is recorded graphically in figure 5, *C* at *a*.

Nicotine. It will be recalled that this drug acted on the frog's lung in a manner similar to section of both vagi. We furthermore showed that in this animal the drug acted by paralyzing the inhibitory center in the medulla as well as the vagus inhibitory terminations in the lungs; for we found that after nicotinization stimulation of the peripheral end of the vagus gives rise to a lung contraction instead of the inhibition seen before giving this drug.

The subcutaneous or deep muscular injections of large (5 to 10 mgm.) doses into the normal intact necturus is in 15 minutes followed by general clonic convulsions with an increase in the rate and amplitude of the gill movements. Tetanus of the gills supervenes and active external respiration comes to an end some time before the general

Fig. 6. Water manometer tracings of the intrapulmonic pressure in necturus. Cannula in tip of each lung. Spinal cord cut and destroyed below medulla. Lungs isolated by ventral median incision. Glottis and tracheal sac closed by mechanical pressure through dorsal traction on front legs.

A: Upper record, left lung; lower, right lung; *a*, pithing of brain; *b*, injection of 0.5 cc. pituitrin in 5 cc. of Ringer's solution into heart. Showing inhibition of lung hypertonus by pituitrin.

B: Upper tracing, left lung; lower, right lung; *a*, section of upper mandible exposing anterior end of brain; *b*, pithing of brain; *c*, injection of 0.7 cc. histamine (1–1000) in 5 cc. Ringer's solution into heart. Showing inhibition of lung hypertonus by histamine.

Fig. 7. Axolotl. Water manometer tracings of the intrapulmonic pressure. Spinal cord pithed and destroyed below level of innervation of front legs. Lungs isolated by abdominal incision; cannula in tip of lungs. Glottis open. Spontaneous respiration (quick movements of lever) except where indicated.

A: a, Gentle mechanical stimulation of gills.

b, Gentle mechanical stimulation of skin of front legs.

c, Gentle mechanical stimulation of skin of mandibles.

d, Strong mechanical stimulation (pressure) of toes of front leg.

x, strong attempt at respiration (swallowing).

B: a, Moderate mechanical stimulation of the gills.

b, Gentle stroking of skin of front leg.

c, Strong mechanical stimulation of the gills.

Showing lung contractions following spontaneous respiratory movements and on stimulation of various sensory nerves.

convulsions cease. The lungs of such animals were found contracted. *Pithing the brain, stimulation of the vagi themselves, or stimulation of the lung itself at its base were without effect.*

If the dose of nicotine injected subcutaneously is reduced still further (2.5 to 1 mgm.) the same symptoms appear in 10 to 15 minutes but are less severe. Pithing of the brain or stimulation of the vagi is again without effect. Direct stimulation of the lung at its base with a strong tetanizing current gives rise to an inhibition of the lung without any or with but feeble return.

From these results it would appear that *a*, the lungs of this amphibian is supplied only with inhibitory fibers through the vagi; *b*, that nicotine paralyzes the respiratory center for the lungs and also the junction between the preganglionic fiber endings and postganglionic cell body, since external respiration ceases with the lungs in hypertonus and since stimulation of the base of the lungs will still yield inhibition when stimulation of the vagus nerve itself gives nothing; *c*, the vagus nerve contains no motor fibers for the pulmonary musculature. Without commenting at this time on the possible significance of this fact, we call attention to the fact that in this most primitive lung which we have studied, the lung is solely under the tonic influence of inhibitory fibers carried by the vagi and motor fibers are apparently absent. We have been unable to elicit a motor response of the lung either by stimulation of the vagi or the lung itself in any normal or nicotinized animal.

The apparent resistance of the peripheral lung mechanism to this drug compared with that of frog may possibly be due to the rather sluggish and imperfect circulation through the lungs.

2. Axolotl. Earlier in this paper we called attention to the fact that the axolotls with which we worked, although possessing gill remnants, were essentially air-breathing animals; and that the lungs were decidedly better developed for that purpose than the lungs of the necturus.

a. Lung contractions at the end of normal respiration. Records were taken of the changes in the intrapulmonic pressure occurring during normal respiration with the glottis open. It can be seen from figure 7, *B*, that every spontaneous respiratory effort (gulp) as indicated in the tracing of the quick movement of the lever is followed by contraction of the lung. These lung contractions compare favorably with those obtained under similar experimental conditions from the frog.

b. Reflex lung contractions. *Gentle* mechanical stimulation applied to the skin of the mandible, gills or front legs induced lung contractions of reflex origin as is seen in figure 7, *A*, at *a*, *b* and *c*. In these three

instances the lung contraction appeared without a preceding attempt at respiration. Subsequent attempts at respiration were followed in each instance by lung contractions in every way similar to those of reflex origin induced by gentle stimulation. It is obvious that the contractions are not the result of changes in the intrapulmonic pressure due to movements of the animal during external respiration; for they occur in the absence of all visible movement of the head region.

Fig. 8. Axolotl. Water manometer tracings of the intrapulmonic pressure. Spinal cord transected below medulla and pithed posteriorly. Cannula in tip of lungs, lungs isolated by median abdominal incision. Glottis closed by forceps. All injections intravenously (in 2 cc. Ringer solution).

A: Lower tracing, right lung; upper, left lung.

a, Transection of upper mandible exposing anterior end of brain.

b, Pithed brain.

c, Injection of 1 cc. 1:10,000 adrenalin.

B: a, Pithed brain.

b, Injection of 0.01 cc. 1:1000 histamine.

c, Injection of 2 mgm. nicotine.

Showing the inhibitory action of these drugs on the lung hypertonus following destruction of the brain, the latter being equivalent to section of the vagi nerves.

Strong mechanical stimulation of the fore legs or gills may lead to partial or complete opening of the glottis with partial or complete collapse of the lungs as is illustrated in figure 7, *A*, at *d*, and figure 7, *B*, at *a*, *b* and *c*. Strong attempts at respiration after marked stimulation of pain fibers as at *xxx* in figure 7, *A*, were not followed by the filling of the lungs until considerably later. It might be assumed that the strong stimulation interfered temporarily with the central control of the mechanism normally at the service of the animal in filling its lungs

with air or depresses the central inhibitory control over the lungs. If the latter is the correct interpretation the animal failed to fill its lungs in spite of violent respiratory attempts because of their hypertonic state. Although we have no direct evidence on this matter the latter interpretation seems to be the more probable one.

c. The central inhibitory control of the lungs through the vagi. It is certain that in the axolotl the medullary centers exert, under normal conditions, a tonic inhibitory control over the lungs as in the frog and necturus; for as a result of the destruction of the medulla which is equivalent to section of both vagi (as in fig. 8, *A*, at *b*) both lungs go into a state of hypertonus.

d. Action of certain drugs on the hypertonic lungs. Adrenalin. This drug temporarily inhibits the hypertonus of the lungs resulting from destruction of the medulla as seen in figure 8, *A*, at *c*.

Histamine. In axolotl the intravenous injection of 1 mgm. of histamine-HCl caused inhibition of the lung musculature not unlike the action of this drug in the hypertonic lung of necturus (fig. 8, *B*, at *b*).

Nicotine. The invariable effect of nicotine on the hypertonic lung of the axolotl is inhibition (fig. 8, *B*, at *c*).

As previously noted we were not only restricted to a few animals in study of this form but found that the physiological state of the animals rapidly declined as the result of our operative procedures. The axolotl furnishes a less hardy physiological preparation than the frog or necturus. As far as our results go they check with those obtained from the frog and necturus.

SUMMARY

1. The vagus center exerts a tonic inhibitory control over the lungs. Destruction of the medulla releases the lung from this control. As a result, the lungs assume a state of more or less permanent hypertonus (necturus and axolotl).

2. Electrical stimulation of the peripheral end of the vagus nerves causes a temporary inhibition of the hypertonic state of the lungs, only on the side of stimulation, during and for some time after the stimulation (necturus and axolotl).

3. The efferent vagi fibers to the lungs are solely of the inhibitory type. We have never seen the slightest indication of a motor response on stimulation of these nerves. Unlike the frog, the vagi of these forms of amphibian life possess few if any motor fibers for the lungs (necturus and axolotl).

4. Electrical stimulation of the base of the lung yields the same results as stimulation of the peripheral end of the vagus (necturus and axolotl).

5. We have been able to elicit reflex lung contractions from gentle mechanical stimulation of cutaneous nerves only in the axolotl. Intense stimulation of sensory nerves (pain) probably prevents filling of the lungs during attempts at respiration because of a hypertonic condition of the lungs due to an inhibition of the inhibitory center.

6. There may appear as a result of destruction of the medulla or strong faradization of the base of the lung a tonus rhythm in the denervated lungs of the necturus. This rhythm has not been seen in axolotl.

7. Adrenalin, pituitrin, histamine and nicotine cause a marked inhibition of the hypertonic condition of the lungs resulting from the destruction at the medulla. Histamine injected into the animal after nicotine causes a contraction of the lung. In axolotl these drugs act in the same direction. Pituitrin was not used in this form; nor was histamine injected after nicotinization of the animal.

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CHANGES IN ACID AND ALKALI TOLERANCE WITH AGE IN PLANARIANS

With a Note on Catalase Content

JOHN W. MacARTHUR

From the Hull Zoölogical Laboratory, University of Chicago, and the Department of Biology, University of Toronto

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During a study of the effects of certain typical acids, bases and salts upon living planarians (*Planaria dorotocephala*, *P. maculata* and *P. velata*), observations were made which are here reported on account of the wider interest of their bearing upon tolerance of H^+ and OH^- ions, upon the relative efficiency of the mechanism for regulation of neutrality in young and old individuals, and upon the problem of acidosis in general.

Methods. A closely graded series of concentration of the acids (hydrochloric chiefly, also sulfuric and acetic) or alkali (sodium hydroxide) is made up from standardized N or 0.1 N solutions by dilution with aerated well water or other (Lake Michigan or Lake Ontario) water in which the worms live and thrive. The well water, which was used chiefly, has a pH value of 7.5 to 7.6 and an ion and gas content to be published with the larger study above mentioned. Both distilled water and strongly chlorinated tap-water are in themselves injurious to these worms and hence could not well be used for the purposes of these experiments; but similar tests are now being made with *P. maculata* in distilled water, this species being but little affected by distilled water in the period of time required.

Into the series of dilutions of an acid or alkali in 500 cc. or 1000 cc. Erlenmeyer flasks, filled and ready to be plugged with rubber stoppers, are introduced the flatworms, usually ten larger (18 to 20 mm.) and ten smaller (8 to 12 mm.) specimens together, all selected sound from established well-fed cultures. In some cases a similar group of three easily distinguishable sizes was used (22± mm., 15± mm. and 8± mm.). In control flasks all such individuals live practically indefinitely.

The hydrogen ion concentrations, already in a graded series from the method of diluting the normal solution, are measured and corrected at critical points by the colorimetric method with appropriate indicators: thymol-blue, brom-phenol-blue, methyl-red, brom-cresol-purple, phenol-red and phenol-phthalein, and the Hynson, Westcott and Dunning apparatus (1). Certain difficulties were encountered from the fact that glassware requires particularly thorough cleaning after each usage, and because strong acids added to water containing so much carbonate as do these naturally generate CO_2 and such solutions tend to return gradually toward neutrality. But these facts have no great significance here except as they render impracticable a precisely accurate determination of the actual limits of tolerance, as may be made in distilled water and by electrometry. In all cases it was the relative rather than the absolute susceptibility that was sought and that is here emphasized.

Extensive physiological studies of planarians have been made by Child (2), (3), who showed for *P. dorotocephala* by various means that the smallest worms (up to about 6 or 7 mm.) consist of but one zoöid, while the medium-sized ones (12 to 14 mm.) usually possess a second zoöid region, and larger specimens (20 to 25 mm. or more) exhibit at the posterior end a third zoöid or group of very small zoöids, constituting what is essentially a "growing tip." At least the chief, the second and the third of these zoöids were demonstrable physiologically by both "direct" and "indirect" methods in cyanides, but only after fission do the typical head structures of the zoöids become visibly differentiated morphologically. The growing tip is evidently involved repeatedly in the reproductive process; hence as this region grows its zoöids become more independent and acquire a higher rate of metabolic reaction and, like young individuals, are more susceptible to high concentrations and less susceptible to low concentrations of lethal agents (KNC, anesthetics, etc.).

Experimental. The chief observations are recorded and summarized graphically in the accompanying figure. The time records are averages from repeated tests made of each dilution; such averages for separate tests show but small deviations from the general average, but individual deviations are often large and overlapping wherever the curves lie close together. Dr. C. M. Child, to whom the writer is indebted for many opportunities and suggestions in this work, has recently used these experiments as part of a class course at the University of Chicago.

Acids. Immersed in HCl solutions that kill almost instantly (e.g., in acidities greater than about pH = 2) all worms are fixed and preserved intact (range of preservation). In lower concentrations, from

pH = 2 down to about pH = 4.5, all individuals are killed and caused to disintegrate, the older somewhat later and more slowly than the younger (range of direct susceptibility and inhibition). In this disintegration all regions of the body are not equally and simultaneously involved, but usually the posterior tip and the head are first attacked

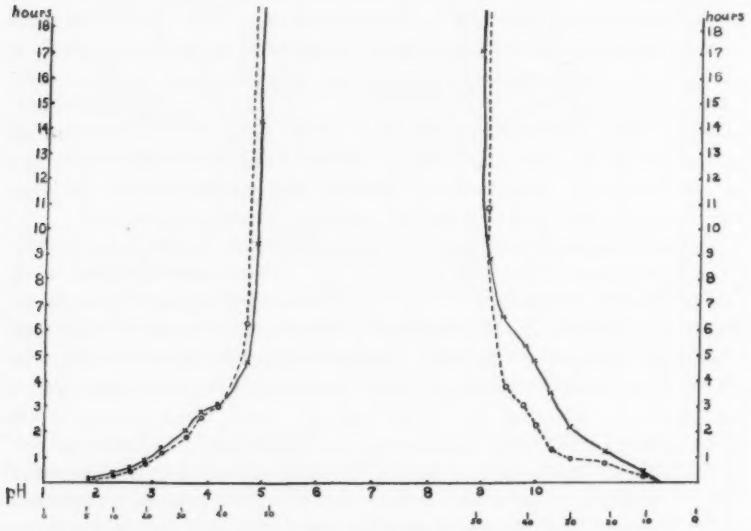

Fig. 1. Time in hours of distinct initial disintegration of young worms - - - o - - - o - - - and old worms ——x——x—— in solutions of different pH values (abscissae) up to pH = 10. Small numbers below abscissae indicate volumes of well water added to one of N/10 HCl (left) or NaOH (right).

Below pH = 2 ± is the range of preservation or fixation. Then follows with increasing dilution up to pH 4.4 ± the range of distinct direct susceptibility which grades off by a transition range into the range of acclimation or indirect susceptibility.

With NaOH the range of preservation is evidently absent, direct susceptibility differences between young and old much more marked, and the indirect susceptibility differences less marked than with acids.

and then the regions behind the head in order from in front backward; and, as might be expected from the small differences in direct susceptibility between young and old, the anterior end of a second zoöid is not distinguished. As the acidity approaches pH = 4.5 or pH = 4.6, the age difference in survival time decreases more and more and finally

becomes nil, and disintegration does not discriminate between the small and the large. In still more dilute solutions, however (pH = 4.7 to 4.9), where death is delayed for several hours and a certain amount of recovery from the initial inhibitory effects of the agent is possible, the relative susceptibility of young and old individuals is reversed (range of indirect susceptibility and acclimation), and smaller worms disintegrate last or not at all, while larger ones either disintegrate entirely or lose their head region. Posterior zoöids are left intact, and the posterior part of the first zoöid was never seen to disintegrate before the anterior end. Often the young worms live for days or indefinitely after the old are partially or wholly gone. Recovery tests, made by returning young and old alike to fresh water after various periods of exposure to the agent, are even more delicate in their indication of the sites and degrees of injury, and were used to extend and confirm the results obtained by leaving the animals in the agent up to the end of the experiment.

A more or less definite sequence of changes leads up to the final disintegration with acids. After the initial stimulation, during which the flatworm assumes for a time a slender form, moves rapidly and secretes some mucus, it gradually loses its power of adherence to the glass walls of the container and becomes shortened, cylindrical and swollen. This state is soon followed by discoloration or whitening, the loss of color occurring, as noted, first at the sensory tip, margins and ventral surface of the head and gradually extending backwards, often more rapidly on the ventral surface, and in larger individuals beginning early also at the growing tip. The whitening appears to indicate that semipermeability or some similar property of the surface layer has been abolished at the approach of death, for following close upon the loss of pigment occur disintegrative changes of a characteristic kind: as the parts become sticky and adherent to glass the regularity of the external contour is interrupted by small breaks in the continuity of the surface, the protoplasmic granules swell and mass into small clumps or liquid spheres and scatter out into the medium until finally little remains of the old body but a soft white shreddy outline composed of the more resistant connective and supporting tissues quite stripped of all the relatively susceptible epithelial parts.

Results differ in no essential way if sulfuric acid in slightly higher concentrations be substituted for the hydrochloric, or if a considerably greater strength of acetic acid be used—the difference probably being necessary to compensate for the lesser dissociation of the organic acid.

A *change of response* occurs upon addition of acid to the normal medium. The planarians then exhibit a fairly strong negative geotropism, climbing always up the walls of the container, whether in doing so they approach or attain a surface or not. Since strong acids cause a release of CO_2 into the solution, the response may perhaps be considered generally appropriate and adaptive, inasmuch as ordinarily an increase of CO_2 is doubtless associated with an insufficiency of oxygen (to which a similar response is made) and both could doubtless be avoided by rising to a better aerated surface layer.

Alkali. In alkaline solutions the same general results are obtained with certain more or less significant modifications. In the hydroxide (NaOH) stimulation is evidently more marked than in acids, and both whole worms and surviving parts of any size are more active both spontaneously and upon mechanical stimulation up to the very point of death. Alkalies also cause the secretion of a very excessive amount of mucus, which collects, as often as removed, in the bottom of the vessel.

Even quickly killing concentrations do not produce a definite fixation and preservation. Disintegration, if rapid, occurs, by a rather violent process of splitting and bursting of the dorsal surface in darkened lines; if slow, it begins at the margins and dorsal surfaces of the head and the posterior tip, and in larger specimens may also appear at what is presumably the anterior end of the second zoöid.

Results with NaOH differ from those with HCl and resemble more nearly those with KNC in one respect—in the slowly acting concentrations, allowing partial acclimation of the larger animals, death sometimes begins at the posterior end of the first zoöid and proceeds forward, while the head region of the first zoöid and all of the posterior zoöids remain intact for some time or indefinitely. In short the details of disintegration with this alkaline agent resemble those with most "acid dyes," while "basic vital dyes" rather resemble acids in their effect (unpublished work).

By the method of direct susceptibility there is much greater difference in survival time of young and old with NaOH than with HCl, the old surviving about twice as long as the young. The effective range of concentrations for indirect susceptibility, on the other hand, is less extended than with acids.

It will be noted that the range of critical concentrations, within which young animals and young parts only are able to regulate slight H^+ ion alterations, is a comparatively limited one and lies just beyond the limits resisted by all alike. Thus increase of H^+ ion up to pH =

4.9 on the one side or of OH^- ion to about pH = 9.1 on the other come within the normal range for all members of the species; slight additional changes (from pH = 4.9 to pH = 4.8 or 4.7 and from pH = 9.1 to pH = 9.2 or 9.3) can be met by the young individuals and parts alone; still greater changes are beyond the powers of acclimation of any, though the old resist the longer.

The greater tolerance by younger planarians and the posterior zoöid region of such dilute acid and alkaline solutions is almost certainly only another example of the greater power of acclimation to mildly depressing conditions associated so generally with more active metabolism (3). In fact the general principle underlying the indirect susceptibility method is founded on the discovery that organisms or parts of organisms possessing an intenser metabolism can acclimate or acquire tolerance more quickly and more completely than less active organisms or parts to low concentrations of cyanides, narcotics, etc. Child also showed later that the anterior, ventral and median regions (the regions of high direct susceptibility and presumably of most rapid metabolism) in Echinoderm and Annelid embryos, developing in low concentrations of NaOH, alcohol or HCl in sea-water, acclimated or acquired tolerance, or after temporary exposure recovered most quickly and underwent a proportionately accelerated and increased development in the larvae (4).

The explanation of this power of acclimation is not known, but may be in some way associated, as regards acids and alkalies, with differences in protoplasmic conditions, such as the higher percentage water content of metabolically active parts or individuals. If this water carries, as seems probable, at least an equal proportional and a greater total salt content, such inorganic salts of these as are buffer-acting substances (carbonates, phosphates, etc.) would act here much as in mammalian blood, to increase resistance to additional H^+ and OH^- ions in the medium. Or, if a greater proportion of mid-products of protein metabolism, or more ionized protein, be present during rapid metabolism, then these amphoteric substances may serve as acids or bases according as there are excess bases or acids in the medium. Naturally such buffers and metabolites would be protective only against slightly and slowly injurious concentrations; with higher concentrations the projective action is quickly overcome and the agent may diffuse and act most rapidly in the parts with greatest water content.

The acid or alkali effects may of course be produced through injury to some enzyme or enzymes essential to continuance of metabolic processes. Inasmuch as the almost universally occurring enzyme, *cata-*

lase, may eventually be shown to play some rôle in metabolism generally and in oxidation in particular (5), the writer wishes to record here the results obtained from numerous experiments to determine the catalase content or activity of planarians of different ages. It was found that equal weights of crushed young worms (8 to 15 mm.), maturer worms (18 to 20 mm.), and of very old worms (25 to 30 mm.) liberated in 15 minutes at 22°C. from 1½ per cent unneutralized hydrogen peroxide the following quantities of oxygen respectively per gram weight of tissue: 653.3 cc., 460.4 cc. and 317.6 cc. Without a single exception, in many repetitions of the experiment, the rule was found to hold that *the larger (older) the worm the lower is the catalase content.* It is of interest to note that the oxygen consumption of young planarians has been found to be from 15 per cent to 100 per cent greater than that of old ones (6), showing a higher basal metabolism, just as the Benedict method does for man.

Some significance should be attached to the fact that though there are such large differences in direct susceptibility of young and old with alkalies, these differences are small with acids; while, on the contrary, though the differences by indirect susceptibility are small with alkalies, they are larger with acids. *The young are evidently comparatively and absolutely less resistant to alkalies, but relatively more resistant to acids.* With advancing age there would appear to be a decreased relative resistance to acids and an increased relative resistance to alkalies—a set of changes such as would result from a gradual onset of a state of acidosis and the more or less incomplete oxidation of the larger-acid products left from a state of lowered metabolism. MacNider (7) has shown that as age advances the acid-base equilibrium of mammals is more and more easily disturbed or overtaxed; that uranium nitrate, for instance, is more toxic to the old than to the young and produces sooner in the old a condition of true acidosis (8), characterized by a depletion of reserve carbonates in the blood, etc.; and that the aged, after uranium treatment, received intravenously without injury considerably more alkali than did the young.

For all the species used by Child (4) in controlling form and proportions of developing embryos, he found that "the agents which are most effective in producing the differentially inhibited type of form are least effective in producing the types of form characteristic of differential acclimation, and vice versa," his series being effective in producing acclimation types in the order: HCl, alcohol, NaOH, NH_4OH, KNC. Thus acclimation was rapid in acids and alcohol, slow in NaOH and

NH_4OH, and exceedingly slow in KNC. A similar contrasting physiological effect between HCl and NaOH is here shown by other means for fresh-water planarians.

Comparing allied species with *P. dorotocephala* it may be said that in general *P. maculata* has a slightly wider range of normal tolerance, while *P. velata* is distinctly less resistant to acids and more resistant to alkalies.

SUMMARY AND CONCLUSIONS

1. *Planaria dorotocephala* of all ages used tolerate HCl up to about pH 4.9 and NaOH up to about pH 9.2 in the well water (pH = 7.5 to 7.6) in which they live, i.e., they tolerate a range of pH from 4.9± to about 9.2±.

2. Smaller, physiologically younger, individuals are on the average tolerant of a slightly wider range of hydrogen ion concentration (from pH = 4.7 to pH = 9.3) than are larger, physiologically older individuals, this difference of susceptibility being apparently somewhat greater on the acid than on the alkaline side of neutrality. The young possess a greater power of neutrality-regulation than do the old, explanatory suggestions for which are offered.

3. In concentrations of alkali which kill within a few hours susceptibility is reversed in relation to age, the young being very much more susceptible than the old. In similar concentrations of the acids young specimens are likewise on the average more susceptible, but only slightly more so. In other words, high concentrations of OH^- tend to increase and high concentrations of H^+ tend to diminish differences in direct susceptibility between young and old individuals. This suggests a possible increasing average acidity with senescence and decreasing metabolism.

4. Young planarians have about double the catalase content of old planarians per gram weight of tissue.

5. In acid solutions liberating CO_2, in which worms live for some time, they commonly assume, as in conditions of oxygen deficiency, a negative geotropism of an obviously adaptive nature as a normal means of escape from excess CO_2.

6. These facts indicate the necessity of taking into account the factors of age, size and metabolism in defining range of tolerance to agents and conditions.

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STUDIES ON THE ALKALINE RESERVE OF THE BLOOD OF THE INSANE

NOBUHARU SUITSU

From the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

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These studies were undertaken to ascertain whether or not the alkaline reserve of the blood of the insane shows any significant differences from that found in the blood of normal individuals, and whether or not the blood of the various types of insanity studied vary one from the other in their carbon dioxide combining capacity. During the course of the investigation certain other material was obtained which will be briefly discussed.

The subjects of these studies were patients at the Pennsylvania Hospital, Department for Mental and Nervous Diseases, Philadelphia.

Since Van Slyke, Stillman and Cullen (1) have shown that a slight rise in plasma carbon dioxide tension usually follows eating, the samples of blood to be analyzed were taken at the uniform hour of eleven o'clock in the morning, three and a half hours after breakfast, unless otherwise noted. The blood specimens were drawn from an arm vein with a Record syringe containing a small amount of potassium oxalate. Care was taken to avoid the sucking in of air. After filling the syringe the point of the needle was plunged under paraffin oil contained in a small test tube and 2 cc. of blood expressed and centrifuged. The plasma was then pipetted off and the carbon dioxide capacity determined as described by Van Slyke (2), and Van Slyke and Cullen (3). The determination was carried out two or three times on one and the same sample and the average adopted as the result.

A general comparison of the findings obtained from excited and depressed cases. It is well known that not only mechanical but also psychic activities have great influence upon respiratory and circulatory functions (1), and that the alveolar carbon dioxide tension under ideal normal conditions indicates the level of the blood bicarbonate, but since the alveolar carbon dioxide tension is altered by numerous factors, psychic, physiological and pathological (4), it is not a reliable measure

of the blood bicarbonate except when it is certain that both the mechanical and nervous factors controlling respiration are normal.

With these facts in view an attempt was made to determine whether or not any relation existed between the plasma carbonate and conditions of excitement and depression. The figures in table 1 give the plasma CO_2 capacity in twelve cases classed as depressed and ten individuals exhibiting excitement.

It will be seen that in general terms all the values fall within normal limits and that no significant differences can be observed between the two groups. The mere fact that no differences occur in these two groups

TABLE 1

The alkaline reserve of the blood of the excited and depressed insane

Cubic centimeters of CO_2 reduced to 0°, 760 mm. bound as bicarbonate in 100 cc. plasma

EXCITED CASES	DEPRESSED CASES
64.42	72.08
64.42	69.14
63.95	66.36
63.57	66.36
63.48	66.36
62.08	63.54
60.20	59.74
54.38	59.68
51.24	57.39
45.04	55.94
	52.06
	51.18
Average.....59.28	61.65

of patients permits the supposition that in long-continued excitations a compensatory reaction occurs sufficient to preserve the normal level of the alkaline reserve of the blood, which one would naturally suppose to be lowered by consequence of the increased activity.

The alkaline reserve of the blood during individual changes in mental condition. A confirmation of the general findings that no evident differences are to be found in the alkaline reserve of the blood of excited or depressed insane patients is afforded by the figures given in table 2. These results represent the analyses of the bloods from single individuals at frequent intervals over periods of several weeks during which there occurred a marked change in mental condition with respect to excite-

ment and relative depression. It is seen that in these cases at least there failed to occur any consistent change in the alkaline reserve of the blood accompanying the change from excitement to depression or vice versa. The four individuals studied were all diagnosed as dementia precox cases.

The variability of the alkaline reserve of the blood of the insane from week to week. During the course of these investigations opportunity was afforded to determine the degree to which the alkaline reserve of the blood varies in the individual from week to week in cases where the general trend of mental condition was uniform.

The results are given in table 3. The variability is given by the value for the average deviation calculated for each individual. An inspection of the table shows that although the absolute amounts all fall within normal limits and no significant differences occur, yet there are differences in the degree of variability to be observed in different individuals. These differences, however, cannot be evaluated since the data are insufficient.

Table 4 is a compilation of the analyses obtained from 112 bloods from 51 individuals arranged in the order of their descending value. The figures represent the cubic centimeters of CO_2 reduced to 0°, 760 mm. bound as bicarbonate by 100 cc. plasma. It is seen that the absolute amounts do not exceed the limits usually attributed to normal bloods save at the extremes. The main fact of interest lies in the relatively low variability of this blood factor which is comparable with that obtained for the average deviation of the blood creatinine nitrogen in similar patients (5).

In table 5 there have been arranged the values representing the average amounts, the average deviation, and the range of fluctuation of the alkaline reserve of the blood according to diagnosis. From the data it is evident that no significant differences obtain in the absolute amounts of the alkaline reserve of the blood in the different types here studied. It should be noted, however, that there is a tendency for the variability of this blood factor to be greater in the mentally disturbed than in normal individuals.

The alkaline reserve of the blood taken three and a half and fourteen hours after eating. Since it is often inconvenient to take samples of blood early in the morning and before breakfast a comparison was made of the alkaline reserve of the blood taken three and a half hours after breakfast, and fourteen hours after the night meal. The data are given in table 3.

TABLE 2

The alkaline reserve of the blood of four dementia precox patients during states of excitement and states of depression

NUMBER	CONDITION	CO_2 REDUCED TO 0° 760 MM. BOUND AS BICARBONATE IN 100 CC. OF PLASMA
		cc.
1	Quiet	50.24
	Excited	64.77
	Excited	63.30
	Quiet	59.68
	Depressed	60.56
	Excited	63.48
2	Excited	60.70
	Excited	63.43
	Depressed	68.51
	Excited	61.07
	Excited	74.80
3	Excited	63.30
	Quiet	60.19
	Quiet	57.78
4	Excited	56.71
	Quiet	54.22
	Quiet	52.49

TABLE 3

The individual variability of the alkaline reserve of the blood of the insane as observed from week to week

Cubic centimeters of CO_2 reduced to 0°, 760 mm. bound as bicarbonate in 100 cc. of plasma

SET 1	SET 2	SET 3	SET 4	SET 5	SET 6	SET 7	SET 8	SET 9	SET 10	SET 11	SET 12
58.46	55.34	53.21	70.07	64.30	65.47	62.60	64.12	74.90	59.80	69.20	72.96
58.15	62.79	63.48	72.00	63.42	64.38	63.51	58.90	73.26	65.46	62.49	73.93
66.73	62.40	62.56	75.38	65.30	57.74	65.50	59.80	70.10	62.58	66.79	64.38
65.66	53.92	71.02	75.31	63.36	67.81	61.54	62.56	70.17	63.30	63.30	67.20
63.42	66.31	63.66	74.85								
63.44	64.34	63.42	73.57								
69.14	70.41										
Average....63.45	62.22	62.89	73.53	64.10	63.85	63.29	61.35	72.11	62.79	65.47	69.62
Variability. 4.0	7.0	5.3	2.3	1.2	4.8	1.9	2.7	2.8	2.3	4.4	5.5

TABLE 4

The carbon dioxide combining capacity of 112 bloods from 51 insane and normal individuals

ALKALINE RESERVE	DIAGNOSIS	ALKALINE RESERVE	DIAGNOSIS	ALKALINE RESERVE	DIAGNOSIS	ALKALINE RESERVE	DIAGNOSIS
75.38	M. D.	66.36	M. D.	63.42	M. D.	58.90	D. P.
75.31	M. D.	66.36	M. D.	63.42	N.	58.62	D. P.
74.90	D. P.	66.31	M. D.	63.42	I. M.	58.46	M. D.
74.85	M. D.	65.66	M. D.	63.36	N.	58.40	D. P.
74.80	M. D.	65.50	D. P.	63.30	D. P.	58.15	M. D.
73.57	M. D.	65.47	D. P.	63.30	D. P.	57.78	D. P.
73.26	D. P.	65.38	N.	63.17	D. P.	57.74	D. P.
72.08	M. D.	65.30	N.	63.09	D. P.	57.39	M. D.
72.00	M. D.	64.77	D. P.	62.79	M. D.	56.71	D. P.
71.04	N.	64.42	M. D.	62.60	D. P.	55.94	M. D.
71.02	I. M.	64.42	S. D.	62.56	D. P.	55.52	D. P.
70.41	M. D.	64.38	D. P.	62.56	I. M.	55.34	M. D.
70.17	D. P.	64.34	M. D.	62.44	M. D.	55.10	?
70.10	D. P.	64.30	N.	62.40	M. D.	54.38	M. D.
70.07	M. D.	64.12	D. P.	62.08	M. D.	54.22	D. P.
69.53	D. P.	63.95	M. D.	61.54	D. P.	53.92	M. D.
69.14	D. P.	53.66	I. M.	61.52	D. P.	53.21	I. M.
69.14	M. D.	63.61	N.	61.33	N.	52.97	D. P.
69.12	D. P.	63.57	N.	61.07	M. D.	52.75	M. D.
69.12	N.	63.57	M. D.	60.70	M. D.	52.65	?
68.60	D. P.	63.54	?	60.56	D. P.	52.49	D. P.
58.51	M. D.	63.54	D. P.	60.20	M. D.	52.06	D. P.
68.32	D. P.	63.54	N.	60.19	D. P.	51.24	D. P.
67.88	D. P.	63.51	D. P.	60.03	N.	51.18	?
67.81	D. P.	63.48	M. D.	59.80	D. P.	50.24	D. P.
67.22	N.	63.48	D. P.	59.74	I. M.	50.20	N.
66.73	M. D.	63.48	I. M.	59.68	D. P.	47.76	D. P.
66.36	M. D.	63.43	M. D.	59.68	D. P.	47.61	?

Average, 62.99; Variability, 7.35; Range, 73.38–47.61.

M. D., Manic depressive; D. P., Dementia precox; I. M., Involutional melancholia; S. I., Senile involution; N., Normal.

TABLE 5

The range of fluctuation, average amounts and average deviations of the alkaline reserve of the blood of the insane according to diagnosis

NUMBER OF CASES	DIAGNOSIS	RANGE	AVERAGE AMOUNT	AVERAGE DEVIATIONS
		cc.	*cc.*	*per cent*
15	Normal	47.61–71.04	62.60	6.6
39	Manic depressive	52.75–75.38	64.42	7.3
8	Involuntary melancholy	51.18–71.02	61.03	7.7
47	Dementia precox	47.76–74.90	61.84	8.2

Sets number 7–8–9 are the values obtained after the shorter fast, and sets 10–11–12 those found after the longer period of abstinence in the same individuals respectively. The investigation extended over eight weeks, the first four weeks of which being the period when the blood specimens were taken once a week after breakfast, and the second four weeks being the period when the samples were taken before breakfast.

It is evident that there are no valid or consistent differences in the bloods taken at these times.

SUMMARY

The results of the studies here reported indicate that:

1. The alkaline reserve of the blood of the insane appears to fall within the limits considered normal for healthy persons.

2. There are no demonstrable differences in the absolute amounts of the alkaline reserve of the bloods from excited or depressed patients here studied.

3. The variability of the plasma carbon dioxide combining capacity seems to be higher in the insane than in the small group of normals here studied.

4. No noteworthy differences obtain in the alkaline reserve of the blood taken three and a half and fourteen hours after eating.

I take this occasion to express my appreciation of the courtesy of Dr. Owen Copp in affording me the facilities of the Pennsylvania Hospital, Department of Nervous and Mental Diseases. The work was carried on under the direction of Dr. Frederick S. Hammett, for whose help and advice I am deeply grateful.

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GASTRIC TONUS OF THE EMPTY STOMACH OF THE FROG

Comparative Studies IV[1]

T. L. PATTERSON

From the Hull Physiological Laboratory, The University of Chicago and the Physiological Laboratory, Queen's University

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Sherrington (1) in 1915 called our attention to the reflex postural activity of muscle and nerve as being the main outcome of the functioning of the proprioceptive part of the nervous system for at least the skeletal muscle. He pointed out that the muscle fiber possessed the property of exhibiting different lengths while exhibiting one and the same degree of tension, and that it was not to be regarded as an elastic band. Furthermore, he believes that unstriated muscle, like skeletal muscle, possesses the same properties as is shown by the ease with which the hollow visceral organs, like the bladder and stomach, adapt their size to the volume of their contents and with very little alteration in their intravesical pressure. Under these conditions, visceral tonus is therefore postural configuration. In confirmation of this Hurst (2) found that the relaxation of the rectum was analogous to what Sherrington described as the "lengthening reaction" of the "postural tone" in the skeletal muscles and in the bladder, and which he at an earlier date had described in connection with the stomach and intestine, although he had not actually used the expression "visceral tone." In case of the skeletal muscle the reflex postural action depends normally upon the afferent nerve of the posturing muscle itself, while in the unstriated muscle it is far less dependent on the central nervous system for its adjustment and maintenance.

More recently Grey (3) has shown by slowly filling the empty viscus with warm physiological saline solution and recording the fluctuations in the intragastric pressure that the normal stomach in rabbits and

[1] A preliminary report of this work was made before the 1919 meeting of the American Physiological Society at Baltimore, a brief abstract of which was published in the Proceedings of that society.

cats is capable of adapting its size to the volume of its contents with very small changes in the intragastric pressure. According to this investigator, the mechanism involved in the postural configuration of the stomach is situated in the wall of the viscus itself and concerns solely its musculature together with its intrinsic nervous mechanism, while the extrinsic nerves exhibit no direct influence, but serve rather to regulate the tension of the stomach wall.

The experiments summarized in this report were undertaken with the view of securing further data on the gastric tonus (postural activity —Sherrington, Hurst, Grey) of the neuro-muscular apparatus as applied to the empty stomach. While the term "postural activity" is very applicable to the skeletal musculature it appears to me that it is not well suited for the unstriated musculature which makes up the larger portion of the walls of the hollow visceral organs, therefore the older and simpler terminology of gastric tonus will be used throughout this paper. The results tend to show that the extrinsic nerves exert a partial influence on the tonal activity of the stomach viscus, as well as serving to modify and regulate the gastric activity at least in the frog. This animal is particularly adapted for such a study for it has been shown in a previous paper (4) of this series that the gastric hunger contractions show no periodicity and no appreciable change in gastric tonus, both features of which are present in the higher animals. In contradistinction to the higher animals, the contractions are practically continuous with scarcely any distinction between the digestive peristalsis and the hunger movements.

Among the first to make observations upon the internal pressure of the hollow visceral organs were Mosso and Pellacani (5) who investigated the bladder in man and in the dog. These authors found that the bladder is capable of adjusting its cavity-volume to different quantities of content, which it enfolds with about the same light tension of grasp whether the viscus is nearly empty or well filled. Somewhat similar observations have been made upon the fundic portion of the stomach. Kelling (6) found that within certain limits the intragastric pressure remained unaffected by the quantity of fluid within the viscus and that the intra-abdominal pressure altered very little in the dog before and after the taking of a copious meal, although the intake of the volume of food might amount to 50 per cent of the total contents of the abdomen in the fasting condition. He infers from these latter observations that the additional volume of contents must be accommodated for by a reflex adjustment of the postural contraction of the

abdominal muscles. Pike and Coombs (7) in confirmation of the above have reported that the introduction of fluid into the stomach or into the peritoneal cavity of cats causes lengthening of the rectus abdominis muscle while the flow of fluid out of the stomach causes a shortening of the same muscle. These changes in the length of the muscle are small and do not occur if the posterior roots of the spinal nerves supplying the muscle have been cut, or if the spinal cord has been transected at the level of the lower cervical roots. The section of both vagi has no marked effect on the response of the muscle. The authors regard the change in the length of the muscle corresponding to the increase or decrease in volume of the contents of the abdominal cavity as a reflex process dependent upon afferent impulses which falls into line with other known instances of postural activity of muscle and nerve. The observations of Sick and Tedesko (8) and others have shown that the gradual filling of a cat's stomach is not accompanied by a rise in intragastric pressure and that the excised stomach, kept alive in a bath of warm oxygenated Ringer's solution also exhibits the same phenomenon to an unmistakable extent.

Cannon and Lieb (9) have also brought forth evidence that each passing of the cardia by swallowed food is accompanied by a rapid small dilatation of the fundus, and that this dilatation is a reflex operated through the vagus. Rogers (10) has reported that central stimulation of one vagus nerve with the opposite nerve intact in the decerebrate dog and after complete splanchnic section leads to reflex spasmodic contractions of the entire stomach and increased gastric tone. Therefore it would appear that the adaptability of the normal stomach at all times is a form of receptive expression brought about by changes in the intragastric pressure as the volume of its contents slowly increases or decreases.

Experimental procedure. The same general method of experimentation was used in the following experiments on the bullfrog (Rana catesbiana) as that described in the preceding paper (11) of this series, with the exception that the gastric balloon was inflated with a known quantity of air by means of a graduated glass syringe sufficient to maintain a constant pressure of 2 cm. in the water manometer and the number of cubic centimeters of air necessary in the different experiments to produce this constant pressure was recorded. Normal contractions of the empty stomach were obtained from each animal, extending over a period of several days, and then these animals were either vagotomized, splanchnetomized or vago-splanchnetomized

(section of both sets of nerves) and the respective observations repeated over a period of from two to three weeks and compared with the normal. The recorded tracings were taken on a slowly moving drum making a revolution in fifty to sixty minutes.

The changes in volume capacity of the stomach as influenced by partial and complete isolation from the central nervous system. The influence of the vagi and splanchnic nerves on the activity of the empty stomach of the frog has been reported in a previous paper (11). According to these results, double vagotomy leads to a sympathicotonic condition of the stomach followed with nearly the normal type of hunger contractions with the exception that they appear to be of a somewhat slower rate and slightly weaker. On the other hand, section of the splanchnic nerves leads to a hypertonic stomach with shallow contractions, showing an increased rate and tending to run into incomplete tetanus, while complete isolation of the stomach from the central nervous system leads to a hypotonic stomach with about the normal type of gastric hunger contractions. Somewhat similar changes have been described by Cannon (12) on cats for the digestive movements and by Carlson (13) on dogs for the movements of the empty stomach.

Although the sectioning of these nerves in various animals has led to certain changes in gastric tonus, as arising from the influence exerted through the extrinsic nerves supplying the stomach, no attempt has been made to analyze the question quantitatively. In order to study the changes in volume capacity of the empty stomach as influenced by partial and complete isolation from the central nervous system, twenty-one animals were used for the various observations recorded herein, as follows,—seven were vagotomized, seven splanchnetomized and seven vago-splanchnetomized. In addition, fifteen other animals were used but as the length of the duration of these experiments was more or less brief due to parasitization or other causes leading to an early death, the data from these were excluded. However, in none of these experiments in which results were obtained were they contradictory to the typical results as tabulated. There were also a few animals of this number excluded because of incomplete nerve section. The following tables have been prepared as showing typical results of the experiments.

The animals used for the observations in the preceding experiments were twelve to thirteen inches in length, extended, and it was found, without exception in this size of animal, that 10 cc. of air introduced by a syringe into the gastric balloon was sufficient to maintain a constant manometric pressure of 2 cm. in the stomach of the normal ani-

mal. Larger animals in proportion to size require greater quantities of air to obtain this constant manometric pressure, and vice versa. In one case, a very large frog measuring sixteen inches, the only one used in the series of experiments, 15 cc. of air were necessary to produce

TABLE 1

*Effect of section of the vagus nerves on volume capacity of stomach and contractions**

DATE 1918	CONDITIONS	AIR IN BALLOON	STRENGTH OF CONTRACTIONS	REMARKS
		cc.	*cm.*	
August 7	Stomostomized			
August 10	Normal	10	6.5	
August 11	Normal	10	6.0	
August 12	Normal	10	6.5	
August 13	Vagotomized			Operation O.K.
August 16	Vagotomized	15	6.5	
August 17	Vagotomized	15	8.0	
August 18	Vagotomized	15	6.8	
August 19	Vagotomized	15	7.0	
August 20	Vagotomized	15	8.0	
August 21	Vagotomized	15	6.5	
August 22	Vagotomized	13	6.5	
August 23	Vagotomized	10	6.0	
August 24	Vagotomized	10	4.5	
August 25	Vagotomized	10	5.5	
August 26	Vagotomized	10	5.8	
August 27	Vagotomized	10	6.2	
August 28	Vagotomized	10	5.0	
August 29	Vagotomized	10	4.0	
August 30	Vagotomized	10	3.5	
August 31	Vagotomized	10	Very weak	
September 1	Vagotomized	10	Very weak	
September 2	Vagotomized			Animal died. Autopsy showed both vagi cut

* Work now in progress on lung tonus of the frog shows that double vagotomy leads to practically the same effects as extirpation of the lungs, and this may shorten the life of the animals.

the constant pressure of 2 cm. in the water manometer. The question of the elasticity of the rubber balloon may arise here for, as Osborne (14) pointed out, in thin-walled rubber bags the extensibility of the elastic material is great and its dimensions, including its thickness, alter much under the stretch imposed. Furthermore, a subspherical

bag may change in general figure as its size is altered, or changes in the physical consistence of the rubber membrane may occur as inflation and deflation proceeds, all of which would lead to serious complication for the analysis of results. However, in the case of the gastric balloon used 10 or even 15 cc. of air do not fill the rubber balloon, so that the tension of the bag's elasticity complicates the stomach tonus.

TABLE 2

Effect of section of the splanchnic nerves on volume capacity of stomach and contractions

DATE 1918	CONDITIONS	AIR IN BALLOON	STRENGTH OF CONTRACTIONS	REMARKS
		cc.	*cm.*	
August 7	Stomostomized			
August 10	Normal	10	6.0	
August 11	Normal	10	6.7	
August 12	Normal	10	6.5	
August 13	Splanchnetomized			Operation O.K.
August 16	Splanchnetomized	4	0.5	
August 17	Splanchnetomized	4	1.0	
August 18	Splanchnetomized	4	0.8	
August 19	Splanchnetomized	4	0.6	
August 20	Splanchnetomized	4	0.5	
August 21	Splanchnetomized	4	1.4	
August 22	Splanchnetomized	6	0.5	
August 23	Splanchnetomized	10	0.3	
August 24	Splanchnetomized	10	0.4	
August 25	Splanchnetomized	10	0.3	
August 26	Splanchnetomized	10	0.3	
August 27	Splanchnetomized	10	0.2	
August 28	Splanchnetomized	10	Very weak	
August 29	Splanchnetomized	10	Very weak	
August 30	Splanchnetomized	10	Very weak	
August 31	Splanchnetomized			Animal diéd. Autopsy showed splanchnics cut

In testing out the amount of air necessary to produce the constant manometric pressure it was found without exception in all the animals that a much smaller amount than 10 cc. of air would produce changes in the manometer amounting to 2 or more centimeters but the length of its duration was very short and the pressure soon fell to the zero level or closely approximated it depending on the quantity of air introduced. This is indicative of the ease with which the stomach adapts its size to the volume of its contents.

In female animals filled with large egg masses the number of cubic centimeters of air necessary to produce the constant pressure showed no variations from that of the non-egg-carrying female and the male, although the abdomen was much enlarged. This condition in the egg-

TABLE 3

Effect of section of the vagi and splanchnic nerves on volume capacity of stomach and contractions

DATE 1918	CONDITIONS	AIR IN BALLOON	STRENGTH OF CONTRACTIONS	REMARKS
		cc.	*cm.*	
October 22	Stomostomized			
October 25	Normal	10	6.5	
October 26	Normal	10	6.5	
October 27	Normal	10	6.9	
October 28	Normal	10	6.7	
October 28	Vagi and splanchnics cut			Operation O.K.
November 2	Vagi and splanchnics cut	15	6.7	
November 3	Vagi and splanchnics cut	15	7.0	
November 4	Vagi and splanchnics cut	15	6.9	
November 5	Vagi and splanchnics cut	15	7.2	
November 6	Vagi and splanchnics cut	15	6.6	
November 7	Vagi and splanchnics cut	15	8.0	
November 8	Vagi and splanchnics cut	15	6.5	
November 9	Vagi and splanchnics cut	15	5.8	Morning
November 9	Vagi and splanchnics cut	13	6.0	Night
November 10	Vagi and splanchnics cut	13	5.0	
November 11	Vagi and splanchnics cut	13	4.4	
November 12	Vagi and splanchnics cut	13	3.5	
November 13	Vagi and splanchnics cut	13	3.4	
November 14	Vagi and splanchnics cut	13	3.5	
November 15	Vagi and splanchnics cut	13	Very weak	
November 16	Vagi and splanchnics cut	13	Very weak	
November 17	Vagi and splanchnics cut	13	Very weak	
November 18	Vagi and splanchnics cut			Animal died. Autopsy showed both vagi and splanchnics cut

carrying female is doubtless accounted for, at least in part, by a reflex mechanism leading to a relaxation of the abdominal muscles, an adaptation similar to the reflex relaxation of the rectus abdominis muscle in increased volume contents of the stomach as has been described by

Pike and Coombs (7). The animals with very few exceptions were run continuously as soon as recovery was complete after the operation and the fast commenced immediately.

Section of both vagi or the vago-sympathetic nerves (11) in the neck of the frog increases the volume capacity of the stomach temporarily, as shown in table 1, from the normal of 10 cc. to 15 cc. of air. This condition invariably lasts from eight to nine days. Usually on the ninth day following the cutting of the nerves there is a decrease in the

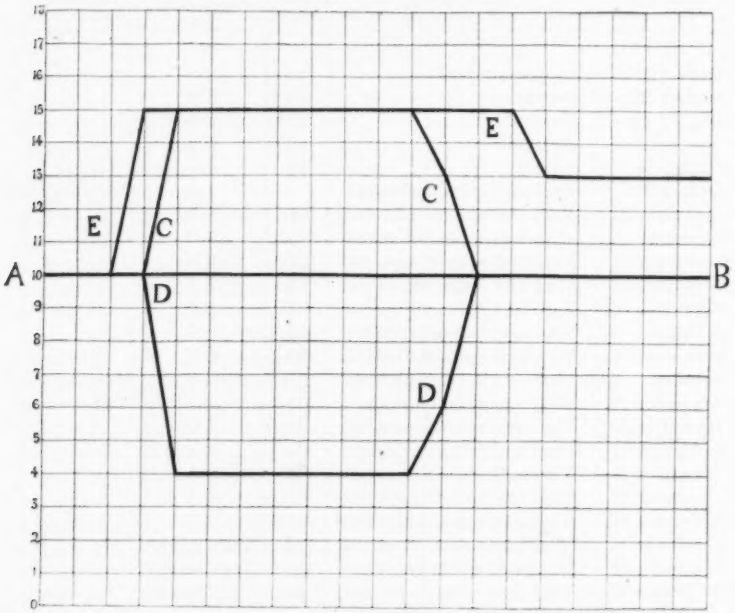

Fig. 1. The spaces left to right indicate the number of days experiment ran. Vertical spaces above and below the heavy line *A B*, representing the normal pressure of 10 cc. of air necessary to maintain a constant pressure of 2 cm. in the water manometer, indicates the positive or negative changes from the constant in the volume capacity of the stomach as influenced by the extrinsic nerves. Curve *C C*, shows effect of sectioning both vagi on stomach. Curve *D D*, effect of splanchnic section. Curve *E E*, combined effect of section of both vagi and splanchnic nerves. Note complete recovery of gastric tonus in first two cases, while in the latter there is only a partial recovery. Heavy line *A B* also indicates negative effect of decerebration on stomach. Figures at left indicate number of cubic centimeters of air in balloon.

intragastric pressure to about 13 cc. of air and on the next day it drops again to the normal or 10 cc. level, and remains there. In other words, the normal tone of the stomach has been reëstablished (fig. 1), and this condition as it exists in the frog may be comparable to the temporary loss of tonus as described by Cannon (12) in cats. The contractions of the empty stomach tend to approach the normal, but on the whole they are of a slightly slower rate and more irregular. The amplitude of the individual contractions may even appear greater than normal and this may be because the contractions start rather suddenly and without any marked preliminary increase in tonus in the fundic end of the stomach.

Section of the splanchnic nerves in the frog markedly decreases the volume capacity of the stomach temporarily, as shown in table 2, from the normal of 10 cc. to 4 cc. of air. This marked diminution in size like the increase after double vagotomy invariably lasts from eight to nine days. Usually on the ninth day following the cutting of the nerves there is an increase in the intragastric pressure to about 6 cc. of air, while on the next day it reaches again the normal or 10 cc. level and remains there. Here again the stomach has reëstablished its gastric tonus (fig. 1). This condition in the frog is much more marked than Carlson (13) found it to be in dogs for just as the number of cardio-inhibitory fibers vary in the vagus of the cat and the dog, so also may not the number of motor fibers in the vagi destined for the stomach vary in different animals? The contractions of the empty stomach are small, showing an increased rate and a tendency to approach incomplete tetanus. This is especially true during the temporary period of high tonal activity when only 4 cc. of air are required to maintain the constant manometric pressure and in one animal 3 cc. of air were found to be sufficient. In a few such animals I have found in the morning following the removal of the balloon the night before such strong gastric and esophageal contraction that it was impossible to introduce the balloon through the short esophagus into the stomach without first introducing a small glass seeker and stretching it. I have even had difficulty in introducing the seeker the first time on one or two occasions because of such marked contraction. This would seem to uphold the views of Cannon (12) and Kelling (6) that the gastric fibers of the vagi function to make the gastric muscles exert a tension.

Section of the vagi and splanchnic nerves in the frog increases the volume capacity of the stomach permanently, as shown in table 3, from the normal of 10 cc. to 15 cc. of air, but in this case there is not

a complete recovery. After this complete isolation of the frog's stomach from the central nervous system, the 15 cc. stomach invariably lasts from twelve to thirteen days, which is a longer period than in either the vagotomized or splanchnetomized stomach. Usually on the thirteenth day following the sectioning of these nerves which is accomplished at one operation there is a fall in the intragastric pressure to a 13 cc. level, where there are no further changes (fig. 1). This new and partial readjustment of the hypotonic stomach is evidently determined by the intrinsic local gastric motor mechanism of the stomach wall for the gastric hunger contractions persist after its isolation from the central nervous system. The appearance of the individual contractions is much the same as when the vagi alone are cut. These contractions may exhibit a greater or lesser amplitude and show a tendency toward irregularity. All the animals in the different groups were autopsied to verify more especially the sectioning of the respective nerves. In a few of these animals in which the heart was still beating regularly the effect of vagal stimulation on the stomach was determined. This resulted usually in a phase of inhibition followed by a stronger phase of excitation immediately upon the removal of the stimulus and is in confirmation with the findings of Hopf (15) on frogs. Stimulation of the sectioned splanchnic usually resulted in a relaxation of the body of the stomach, if any change at all occurred, and if the stimulation was repeated several times in succession it seemed to bring about a constriction of the pyloric sphincter and perhaps also that of the cardiac sphincter, a condition which would seem to indicate that the splanchnics might possess a few fibers of the motor type. The stimulation of these two nerves does show, however, that the fibers of neither have degenerated, and since the rate of nerve degeneration differs in different animals and in frogs requires from thirty to one hundred and forty days, depending upon the season of the year (16), there is no possibility of the regeneration of these nerves. The normal functioning of the two sets of nerves to the stomach is indicated by the results of sectioning, as well as by the results of stimulation. In the case of the isolation of the stomach from the influence of the vagi with the splanchnics intact, or vice versa, there is a perfect physiological readjustment of the normal tonus of the gastric musculature. On the other hand, after complete isolation of the stomach (vagi and splanchnics severed) from the central nervous system there is only a partial physiological readjustment of the gastric musculature. This indicates that the extrinsic nerves play a prominent part in the maintenance of gastric tonus, at least in the frog. When

the splanchnics are sectioned it must be the motor fibers of the vagi that produce the high and temporary gastric hypertonicity, i.e., hypertonic stomach. When the vagi are cut and the splanchnics permitted to exert their full influence on the gastric musculature it is reasonable to believe that these nerves must possess motor fibers probably to the sphincter muscles and that these areas then act as tonic rings which, in connection with the intrinsic or local reflex mechanism of the gastric wall, are capable of producing a perfect physiological readjustment. Whereas, in the case of the stomach completely isolated from the central nervous system, this intrinsic or local gastric mechanism is incapable of bringing about a complete readjustment and in consequence of this it creates a new level of gastric tonus. Thus, every reflex is in its own measure an integral reaction, and is purposive in that it bears some biological purport for its organism. This physiological readjustment occurred regularly in all the animals, that is, it could be looked for after a lapse of a certain number of days following the sectioning of the nerves. For example, in the case of the vagotomized stomach when this readjustment started I have seen in a few instances the pressure in the manometer increase from the constant level of 2 cm. to 5 or 6 cm., but I have never observed it in the stomach of the normal animal. In the splanchnetomized stomach of the course the first readjustment stage is marked by a fall in the manometric pressure to zero.

The changes in gastric tonus observed throughout this series of experiments are so slight in the normal frog that they are practically unmeasurable. However, tonus is the prime condition for that tension which must be developed before contraction can result and if the tension persists the contraction recurs (17). Furthermore, the importance of the tonic state in the normal functioning stomach is reinforced by the fact that when all the extrinsic nerves are cut the stomach develops in time within itself a tonic state, while the adaptability of the abdominal cavity to the volume of its contents is left to the postural reflex.

The effect of decerebration on the tonus of the stomach. It has been shown by King and Connet (18) that the rate of the gastric contractions is increased in decerebrate guinea pigs and that the stomach becomes hypertonic. According to Rogers (19) the hyperactivity of the crop of the decerebrate pigeon is inhibited by food and water as in the normal bird, while the writer (4) has reported no change in the type of the contractions from the empty stomach of the normal and the decerebrate frog. In order to study the effect of decerebration on the volume capacity of the stomach, observations were made on six decerebrate frogs. The following table has been prepared from a typical experiment.

Decerebration in the frog has no effect on either the volume capacity of the stomach or the amplitude of the individual contractions, as is shown in table 4. There is also no change in the type of contractions, which confirms the work of the previous paper (4). The negative findings in these experiments show that the higher cerebral centers in the frog play no appreciable part in either the maintenance of gastric activity or the tonic state. Since section of the vagi leaves the stomach in a temporary hypotonic condition (15 cc. stomach) while the decerebration effects are negative we may infer that impulses from centers

TABLE 4

Effect of decerebration on volume capacity of stomach and contractions

DATE 1918	CONDITIONS	AIR IN BALLOON	STRENGTH OF CONTRACTIONS	REMARKS
		cc.	*cm.*	
July 26	Stomostomized			
July 31	Normal	10	6.0	
August 1	Normal	10	6.5	
August 2	Normal	10	6.0	
August 2	Decerebrated 4:15 p.m.			Operation O.K.
August 2	Decerebrated	10	6.0	Contractions started again at 5:35 p.m.
August 3	Decerebrated	10	6.3	
August 4	Decerebrated	10	6.0	
August 5	Decerebrated	10	5.2	
August 6	Decerebrated			Animal died. Autopsy showed complete removal of cerebral hemispheres

in the mid-brain and medulla exercise the controlling influence and produce after section of the splanchnics the temporary hypertonic stomach, i.e., high gastric tonus. It may be further implied that there is a dynamic readjustment in the central nervous system which leads to an actual diminution in the inhibitory impulses through the splanchnics after vagal section or an inverse motor condition existing through the vagi after splanchnic section, or else the stomach may bring about its physiological readjustment by an increased resistance or tolerance of the splanchnic or motor impulses over the respective nerves to the gastric mechanism.

CONCLUSIONS

1. The normal stomach of the frog possesses a marked capacity for adapting itself to the volume of its contents with only minimal changes in the intragastric pressure. This is in confirmation with the work of Grey and others.

2. Both the intrinsic and extrinsic nerves take part in the maintenance of gastric tonus as is shown by partial and complete isolation of the stomach from the central nervous system.

3. Section of the vago-sympathetic nerves (double vagotomy) with the splanchnics intact increases the volume capacity of the stomach temporarily, but there is later a complete readjustment.

4. Section of the splanchnic nerves with the vagi intact decreases the volume capacity of the stomach temporarily, but there is again a complete readjustment as above.

5. Section of both the vagi and splanchnic nerves (complete isolation from the central nervous system) increases the volume capacity of the stomach permanently, and in this case there is only a partial readjustment, at least for a period extending over three weeks and the tonus of the stomach is established upon a new level from that of the normal.

6. Decerebration affects neither the volume capacity of the stomach nor the type of the contractions.

The writer desires to acknowledge his indebtedness to Doctor Carlson for his kindly and valuable criticism.

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STUDIES ON THE SUBMAXILLARY GLAND

VI. On the Dependence of Tissue Activity upon Volume-flow of Blood and on the Mechanism Controlling this Volume-flow of Blood

ROBERT GESELL

From the Departments of Physiology of Washington University Medical School and of the University of California

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INTRODUCTION

This paper has to do with two problems: one the dependence of tissue activity upon volume-flow of blood, and the other the mechanism by which the volume-flow of blood is controlled. While these problems may be considered as distinct from each other, yet they have a certain interdependence which may warrant their discussion in common.

I have previously reported results bearing upon both problems (1), (2). Although no definite conclusions were reached concerning the mechanism of volume-flow control, it was shown that in hemorrhage the organism as a whole suffers from a reduced flow of blood as is indicated by the reduced alkaline reserve of the plasma of the blood. But despite the fact that the organism suffers from a reduced flow of blood we know from the work of others (3), (4) that a tissue may be stimulated to great activity even though the flow of blood may be very low or even absent. This apparent independence of volume-flow and tissue activity is shown in figures 1 and 3, where secretion of saliva is used as the index of tissue activity.

DEPENDENCE OF SECRETION UPON VOLUME-FLOW OF BLOOD

To show the dependence of tissue activity upon volume-flow of blood, using secretion as the index to activity, special methods must be employed. The greatest care must be taken that change in volume-flow of blood be the only variable. When the gland is activated by stimulation of the chorda tympani the periods and strength of stimulation

must be equal and periods of rest must be chosen which will avoid the augmenting effect of previous stimulation. Results obtained under such conditions can be compared with results obtained when the blood supply is modified. Such results are shown in figures 1, 2 and 3, in which blood pressure, volume-flow of blood, secretion, electrical deflections, time in seconds and moment of stimulation of the chorda tympani are recorded. The volume-flow of blood was measured with the bloodless method previously described.

Figure 1, *A* and *B*, shows the effects of occluding the carotid artery during a short period of stimulation lasting 14 seconds. In the first record, the artery was unoccluded and stimulation of the chorda tympani produced the usual rapid acceleration of blood flow. In the second, where the artery was occluded, the flow of blood during the period of stimulation was slow but the amount of saliva secreted was not diminished. The results might be taken to indicate that even large fluctuations in volume-flow of blood need not affect the metabolic processes of the gland, were it not for the change in the contour of the electrical deflections which suggests that the glandular processes were modified. On the other hand, the absence of an after-flow of blood following de-occlusion indicates that the gland was not overtaxed by the temporary reduction in the blood-flow.

Figure 1, *C* and *D*, shows the effect of occlusion of the artery during greater activation. The secretion of saliva during occlusion was not reduced, yet the electrical deflection was modified again as in 1, *B*. The difference between the results shown in 1, *A* and *B*, and in 1, *C* and *D*, is that de-occlusion in the latter observations was followed by a markedly accelerated after-flow of blood indicative of an overstrain of the tissue resulting from activation without sufficient blood supply.

Figure 2, *A*, *B* and *C*, shows the effect of more prolonged occlusion of the artery lasting through a period of stimulation of 30 seconds. Even this longer period of occlusion has little effect upon the amount of saliva obtained—16.2 drops during the period of occlusion as compared with 18.2 and 19.2 drops during the preceding and subsequent periods of free flow of blood. In figure 3, *A*, *B*, *C* and *D*, where the periods of stimulation and occlusion were still longer, the reduced flow of blood again had relatively little effect. In fact (*C*) during occlusion 18.4 drops of saliva were secreted compared with 19.0, 19.6 and 20.3 in the preceding and subsequent periods.

Upon the whole, the results indicate that relatively short periods of occlusion of the carotid artery affect little the amount of saliva

elicited by stimulation of the chorda tympani. This absence of marked effects may be apparent only and may be due to the fact that the gland at the moment of stimulation has recovered from previous activation and readily liberates its stored material and energy regardless of the momentary decrease in flow of blood during the period of activation. If so, it would be better to study the dependence of tissue metabolism

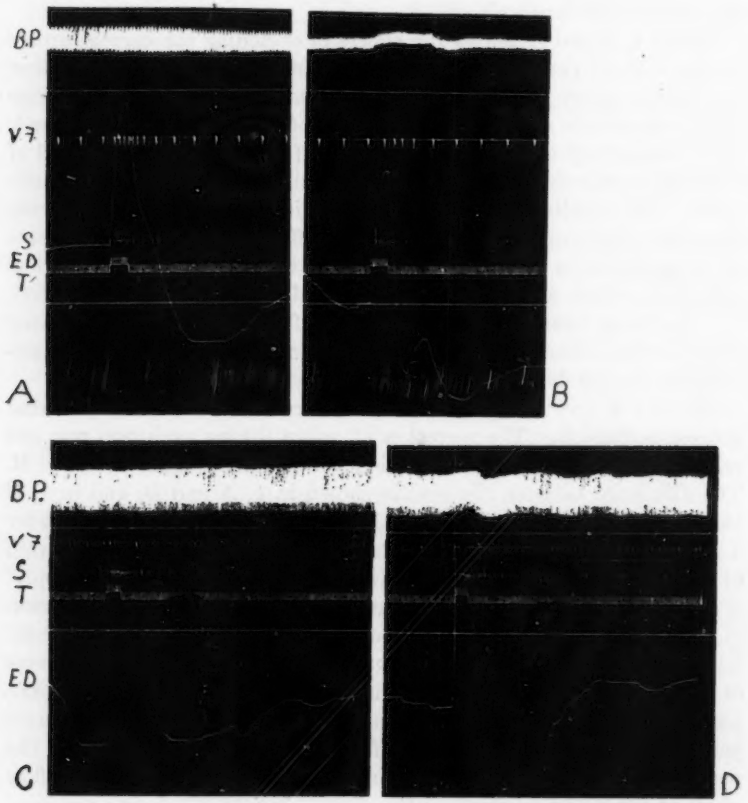

Fig. 1. Effect of occlusion of the carotid artery on the response of the submaxillary gland to stimulation of the chorda tympani. 1*A*, normal; 1*B*, artery occluded; 1*C*, normal; 1*D*, artery occluded; *B.P.*, blood pressure; *V.F.*, volume-flow of blood; *S.*, salivary secretion; *E.*, electrical deflection; *T.*, time in seconds and moment of stimulation of the chorda tympani.

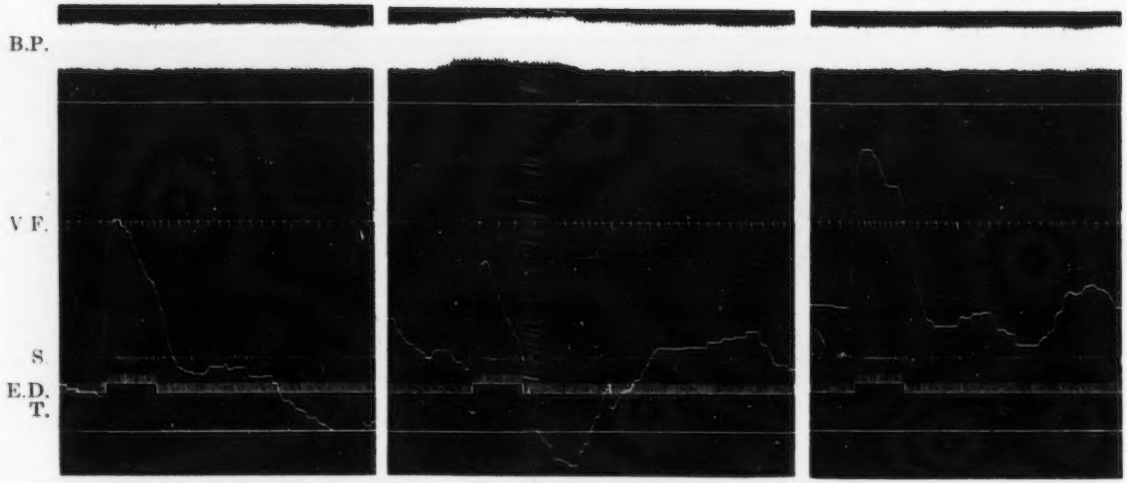

Fig. 2. Effect of occlusion of the carotid artery on the response of the submaxillary gland to stimulation of the chorda tympani. 2*A*, normal, 18.2 drops of saliva secreted; 2*B*, artery occluded, 16.2 drops of saliva secreted; 2*C*, normal, 19.2 drops of saliva secreted.

Fig. 3. Effect of occlusion of the carotid artery on the response of the submaxillary gland to stimulation of the chorda tympani. *A*, *B* and *D*, normal records with 19.0, 19.6 and 20.3 drops of saliva secreted; *C*, artery occluded, 18.4 drops of saliva secreted.

upon tissue which has not fully recovered from previous activation; for this purpose the gland might be activated over a longer period of time, and the blood supply modified during this period of activation, that is while activity and recuperation are going on hand in hand.

A series of such experiments is shown in figure 4, *A*, *B*, *C*, *D* and *E*. In figure 4, *A*, *B* and *C* the gland was activated by the injection of pilocarpin and the flow of blood was restricted in three ways: in *A* by obstruction of the carotid artery; in *B* by injection of adrenin; and in *C* by stimulation of the vago-sympathetic. In every instance the slowing of the flow of blood affected the response of the gland to the stimulation of pilocarpin. The objection might be raised that we not only interfered with the blood supply but also with the supply of pilocarpin which stimulates the gland. This objection can not hold in the experiment represented in figure 4, *D* and *C*, where the gland was activated by stimulating the chorda tympani. In 4, *D*, the flow of blood was decreased during stimulation by occluding the artery. The volume-flow of blood was not recorded but the moments of occlusion and de-occlusion are evident in the blood pressure tracing. It will be noted that the effect of occlusion became progressively greater as occlusion continued, suggesting that stored up saliva may be easily liberated, whereas the storage and liberation of new saliva required a greater flow of blood. The slowing of the secretion is undoubtedly due to the slowing of the blood stream and not to fatigue of the gland from prolonged stimulation, as is indicated by the subsequent acceleration of secretion upon de-occlusion of the artery. In this record secretion continued for some time after cessation of stimulation. Occlusion of the artery during this after-secretion again retarded secretion. The effects of injection of adrenin during prolonged stimulation of the chorda tympani were quite as striking—see figure 4, *E*.

The gland from which figure 5 was obtained was extremely sensitive to changes in volume-flow of blood. The period of stimulation of the chorda tympani lasted from *A* to *B*. The close parallelism between secretion and volume-flow of blood is very evident. Short irregular fluctuations which were not due to occlusion of the artery also are to be seen.

Figure 6 is taken from the same experiment as figure 5. It shows again the effect of volume-flow of blood upon the threshold of stimulation for secretion. Stimulation of the chorda tympani elicited an increased volume-flow of blood and though no visible secretion occurred it excited the gland, as is evidenced by the electrical deflection. That

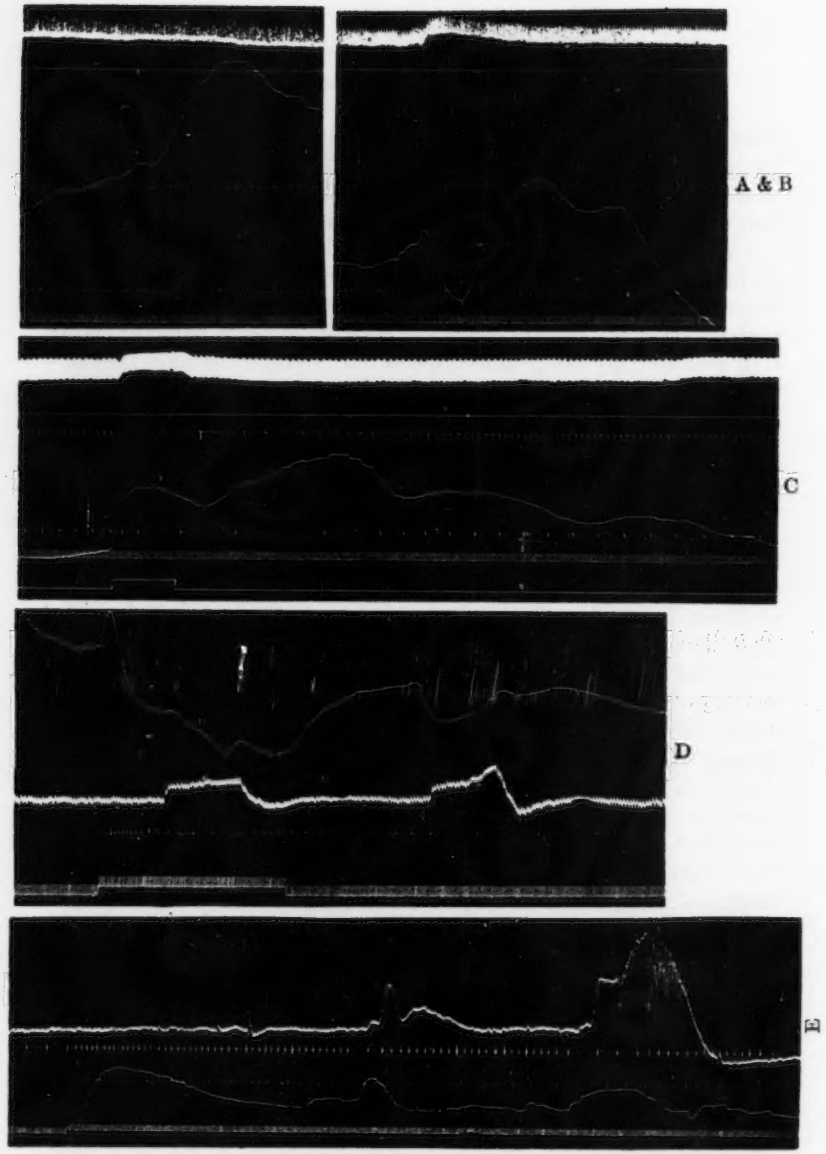

Fig. 4. Effect of interfering with the blood supply to the submaxillary gland during prolonged activity. In *A*, *B* and *C*, the gland was activated by the injection of pilocarpin and the blood flow interfered with by arterial occlusion, injection of adrenin and stimulation of the vagosympathetic respectively. In *D* and *E* the gland is activated by stimulation of the chorda tympani and the flow of blood interfered with by arterial occlusion and the injection of adrenin.

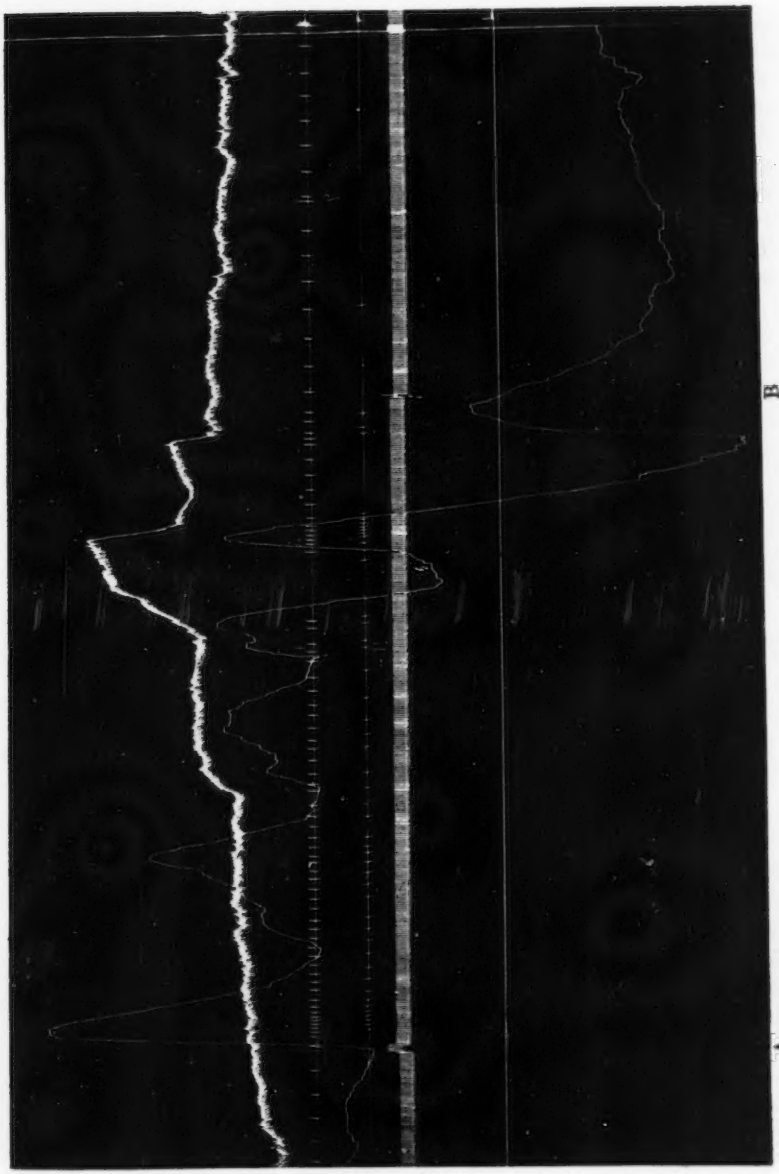

Fig. 5. Relation of secretion to volume-flow of blood.

occlusion of the carotid artery during excitation affected the processes in the gland is indicated by another change in the electrical deflection. When the artery was de-occluded about 20 seconds after the cessation of stimulation a rapid flow of blood occurred. This accelerated blood flow was accompanied by a copious secretion. The fact that an accelerated blood flow was associated with secretion some time after the effects of stimulation had wholly or at least partially worn off, seems to indicate that secretion in this instance occurred in two definite stages. The results are in line with the observation that the elicitation of visible secretion requires a stronger stimulus than does the elicitation of an

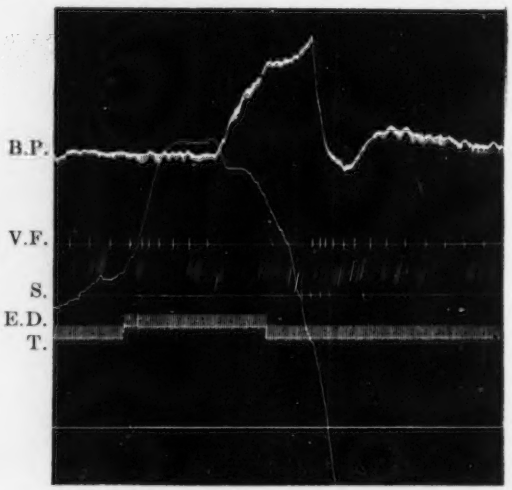

Fig. 6

electrical deflection which is an index to glandular activity. There may, however, be another explanation of the results shown in figure 6. We know from previous work that adrenin, provided it does not interfere with the flow of blood through the gland, may occasionally augment secretion elicited by either stimulation of the chorda tympani or injection of pilocarpin. It is possible that the short period of arterial occlusion led to an asphyxial discharge of adrenin into the circulation (5) sufficient to augment secretion. Unfortunately results similar to those seen in figure 6 were not obtained frequently enough to make possible the determination of the factor underlying the accelerated secretion.

THE MECHANISM CONTROLLING THE VOLUME-FLOW OF BLOOD

It is well known that after atropinization of an animal stimulation of the chorda tympani may accelerate the volume-flow of blood through the submaxillary gland without eliciting visible secretion. This observation is used as evidence of the presence of vasodilator fibers in the chorda tympani. But Barcroft (6) pointed out that even though there be no visible secretion resulting from stimulation of the chorda tympani, oxidations in the gland may be increased. The accelerated flow of blood may, therefore, be due to liberation of dilator metabolites rather than to stimulation of dilator fibers.

The fact that stimulation of the chorda tympani, too weak to produce visible secretion, may elicit an increased volume-flow of blood is likewise cited as evidence for the presence of dilator fibers in that nerve. But the fact that such an increase in volume-flow of blood is accompanied by an electrical deflection also makes possible the explanation of dilatation through dilator metabolites (1).

Since these methods, which indicated the existence of dilator fibers, fail to yield crucial data concerning the mechanism of volume-flow control, I attempted in a previous research to throw further light on the problem by a variety of indirect methods (1). The results obtained were summarized as follows:

> As to the existence of vasodilator nerves the question which initiated this research nothing definite can be said. We have no proof that such nerves do not exist, neither have we proof that metabolites can not adequately control the volume-flow of blood. All that can be said is, that if the dilator fibers do control the volume-flow of blood, this flow may be augmented still more by an accumulation of metabolites. Many observations might apply to both theories, some however point more strongly to the metabolite control.

It was shown in that research that with conditions constant the flow of blood is very finely adjusted to the activity of the gland. By plotting the superbasal flow of blood on the ordinates against progressively increasing amounts of salivary secretion on the abscissas we found superbasal flow of blood to be a linear function of superbasal metabolism. Such accelerated flow accompanying tissue activity is undoubtedly a purposive reaction to make good the excess of liberated energy, but does not help toward determining the mechanism of volume-flow control. This reaction of accelerated flow of blood is studied to advantage by plotting continuous curves of glandular activity and volume-flow elicited by stimulation of the chorda tympani. This method not

only brings out in another way the parallelism of the two phenomena but in addition shows the time relation of the two processes.

Figure 7 shows the effect of stimulation of the chorda tympani of 12 seconds duration in 7, *A*, and of 60 seconds in 7, *B*. The activity of the gland (secretion) is plotted on the ordinates in solid black against time on the abscissas. Volume-flow of blood is plotted as a line. The portion of the curve of volume-flow of blood preceding stimulation of the chorda tympani represents the basal flow of blood or the flow of rest; the curve above that level represents the superbasal flow. Though in each instance the increased flow of blood followed stimulation

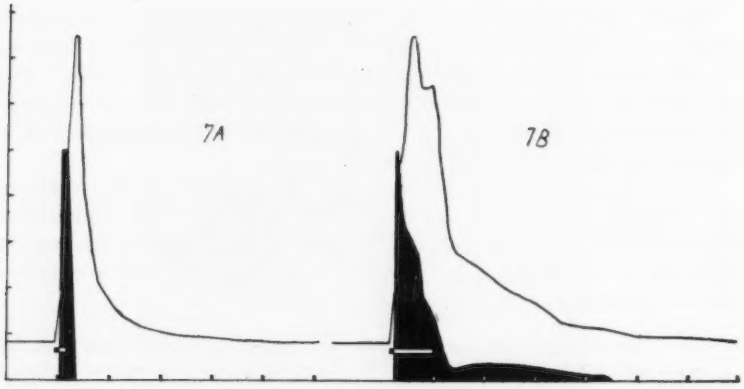

Fig. 7. Effect of stimulation of the chorda tympani on secretion and volume-flow of blood. Volume-flow of blood and salivary secretion are plotted on the ordinates against time in minutes on the abscissas. The duration of stimulation of the chorda tympani is shown.

promptly, there was considerable lag in the flow as indicated by a comparison of the crests of the curves of secretion and volume-flow of blood. This lag or after-flow of blood, which continues throughout the experiment, is suggestive of a recuperative process following activation, and is in agreement with the findings of Barcroft and Hill on oxidation and heat formation associated with tissue activation.

Figure 8 shows results obtained in other experiments. The graphs were obtained from four different animals. Graphs *A* and *B* show the relation of volume-flow of blood to prolonged activity of the gland elicited by prolonged excitation of the chorda tympani. The usual effect of such stimulation upon secretion is a rapid acceleration followed

by a decrease which in turn gives way to a secondary increase. The parallelism between secretion and volume-flow of blood is striking.

Graph 8, *C* and *D*, shows the same relation, but the fluctuations in secretion differ from those in graph 8, *A* and *B*, in that they are elicited by short periods of stimulation. Secretion elicited by stimulation of

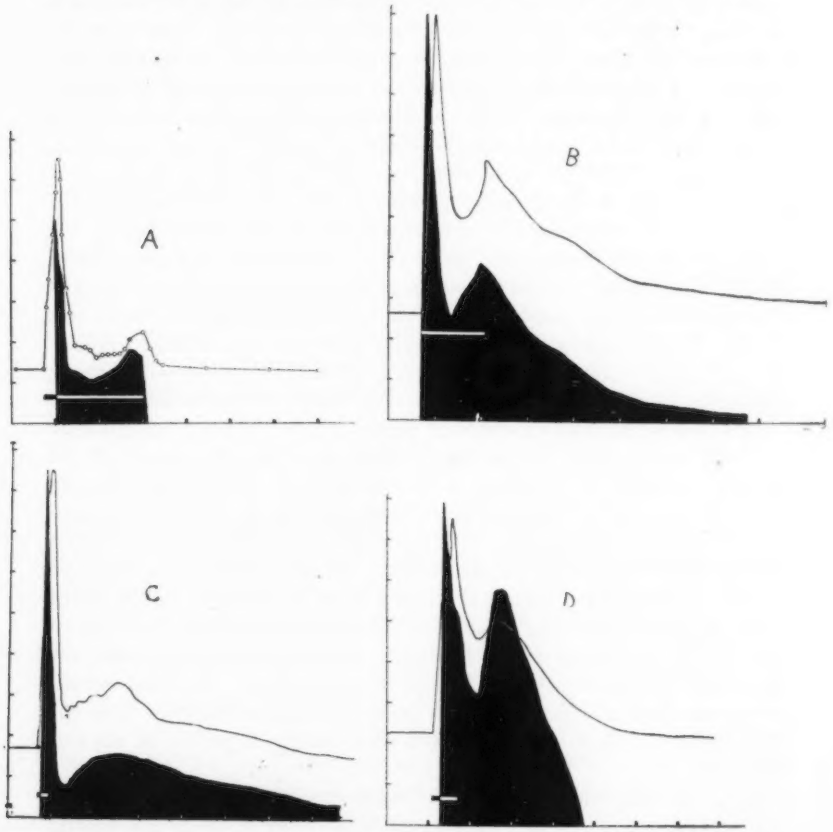

Fig. 8. Effect of stimulation of the chorda tympani on secretion and volume-flow of blood. Volume-flow of blood and salivary secretion are plotted on the ordinates against time in minutes on the abscissas. *A* and *B* show the relation of volume-flow of blood to prolonged secretion elicited by prolonged stimulation of the chorda tympani. *C* and *D* show the relation of volume-flow of blood to prolonged secretion elicited by a short period of stimulation of the chorda tympani.

the chorda tympani as a rule stops promptly upon cessation of stimulation, but not infrequently, the secretion slows only to accelerate before ultimately decreasing. Such results are represented in graph 8, *C* and *D*. The volume-flow of blood here, too, is closely adjusted to the activity of the gland. It is interesting to consider the significance of these results in relation to the mechanism of vasodilatation. The after-secretion following cessation of stimulation may last from 1 to 15 minutes. The cause of this secretion we do not know. It may possibly be due to a prolonged after-discharge of the vasomotor post-ganglionic cells. A like discharge of the vasomotor post-ganglionic cells would explain in a similar manner the after-flow of blood. If both after-flow of blood and after-flow of secretion are due to this after-discharge of ganglionic cells it is extremely interesting that the after-discharge of the two sets of cells should be so nearly equal and so exactly timed.

Numerous experiments similar to those represented in figures 7 and 8 show that under constant conditions and regardless of the duration of stimulation, the volume-flow of blood is nicely adjusted to the needs of the tissues. Although such results need not favor either theory of vasomotor control, the lag of accelerated volume-flow following activation might suggest at least the coöperation of metabolite control.

If a lack of parallelism could be demonstrated between volume-flow of blood and activity of the gland resulting from stimulation of the chorda tympani, the presence of dilator fibers in the chorda tympani would be suggested, only, however, assuming the absence of vasoconstrictor fibers. But it appears to be a difficult matter to demonstrate satisfactorily such a lack of parallelism. Perhaps graph 8, *D*, could be looked upon as representing a slight lack in the perfection of adjustment between volume-flow and tissue activity, in that the second increase of secretion is associated with a relatively smaller increase in volume-flow than that associated with the first phase of secretion. In a few experiments the lack of parallelism has been far more striking in that the volume-flow of blood actually decreased during a period of copious secretion.

As to the significance of these apparent exceptions, it is obvious that the presence of a variable number of constrictor fibers in the chorda tympani might change the relation between glandular activity and volume-flow of blood elicited by stimulation of the nerve. Fröhlich and Loewi (7), believe that such fibers exist. They obtained a decreased flow of blood, such as is described above, when the chorda tympani was stimulated. In their experiments nitrites were administered for the

purpose of producing a maximum dilatation, thereby permitting effective stimulation of the constrictor fibers running in the chorda tympani. Bayliss (8) failed to obtain the decreased flow of blood under the conditions given by Fröhlich and Loewi and furthermore failed to confirm the results by stimulation of the cervical sympathetic nerve of the cat, which is known to contain constrictor fibers.

I attempted to determine the presence of constrictor fibers running in the chorda tympani by selective stimulation of these fibers produced by hemorrhage. I recorded the volume-flow of blood from both submaxillary glands during progressive hemorrhage. On one side the chorda tympani and the vago-sympathetic were cut and on the other side only the vago-sympathetic. Since one gland was connected with the central nervous system through the chorda tympani and the other gland was completely isolated, if constrictor fibers are present in the chorda tympani we might expect a difference in the curves of basal flow of the two glands (basal flow of blood plotted on the ordinates against mean blood pressure upon the abscissas). A comparison of the curves of basal flow of blood failed to indicate the presence of constrictor fibers in the chorda tympani.

The results shown in figure 9 are more helpful in explaining the difference between the results of Fröhlich and Loewi and of Bayliss. In this figure the curve of secretion and of basal-flow of blood and of salivary secretion elicited by short periods of stimulation of the chorda tympani at various levels of blood pressure during progressive hemorrhage are plotted. The period of stimulation in each case lasted about 20 seconds. The curve beginning on the abscissas is the curve of secretion. The other is the curve of blood flow. The horizontal portion preceding stimulation of the chorda tympani represents the flow of rest or basal-flow and the remaining portion the secretory or super-basal flow of blood. Record *A* was obtained during normal blood pressure. In record *D* the pressure had fallen to about 40 mm. Hg.

From the work of Barcroft we know that during secretion water is abstracted from the blood flowing through the gland. As the basal-flow of blood diminishes with decreasing pressure the basal and super-basal-flow of blood decrease and the configuration of the curves changes in a way indicative of this abstraction of water, for it will be noted that in the final stages of hemorrhage the flow of blood during secretion is actually less than the flow of rest. The irregularities of the curves of blood flow in graphs *B* and *C* apparently are the result of abstraction of water from the blood, but only when the blood pressure is too low

to take sufficient advantage of the dilatation which presumably occurs, does the abstraction of water reduce the flow below that of rest. This reduction gives the appearance of constriction.

The exceptions to the proportionality of tissue activity and volume-flow of blood, therefore, can not be said to be real, but figure 10 shows results which may possibly be of significance. This figure shows the effect of reducing in steps the strength of a prolonged and continuous stimulation of the chorda tympani. The period of stimulation lasted

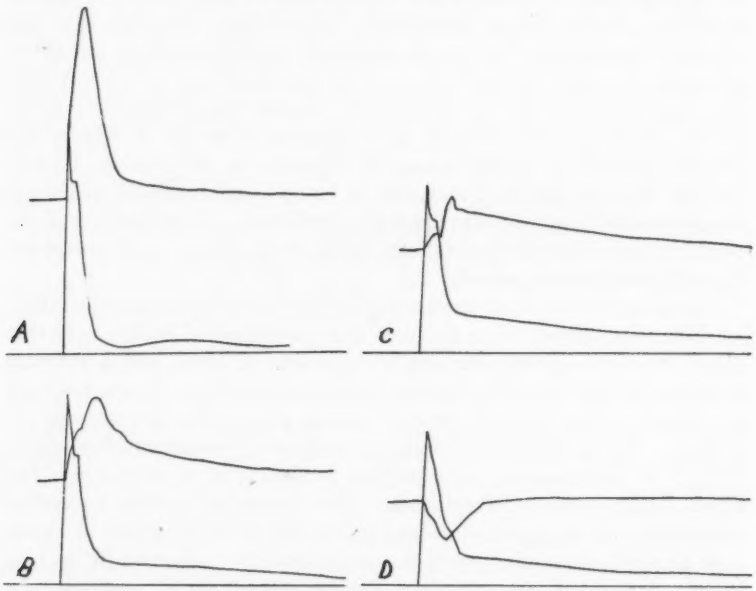

Fig. 9. Relation of volume-flow of blood to secretion during progressive hemorrhage.

approximately 7 minutes. Figure 10, *A*, shows the usual results of such a procedure and figure 10, *B*, the unusual results. In figure 10, *A*, it will be noted that at the points *B*, *C* and *D*, where the strength of current was decreased, volume-flow of blood and secretion showed proportional changes. Not so in figure 10, *B*. For some reason the response of the gland to the same procedure was strikingly different. The chorda tympani was stimulated at *A* and the strength of stimulation kept constant up to *B*. During this period the usual relations between

volume-flow of blood and secretion obtain. At *B* the strength of stimulation was suddenly decreased and, barring the smaller variations

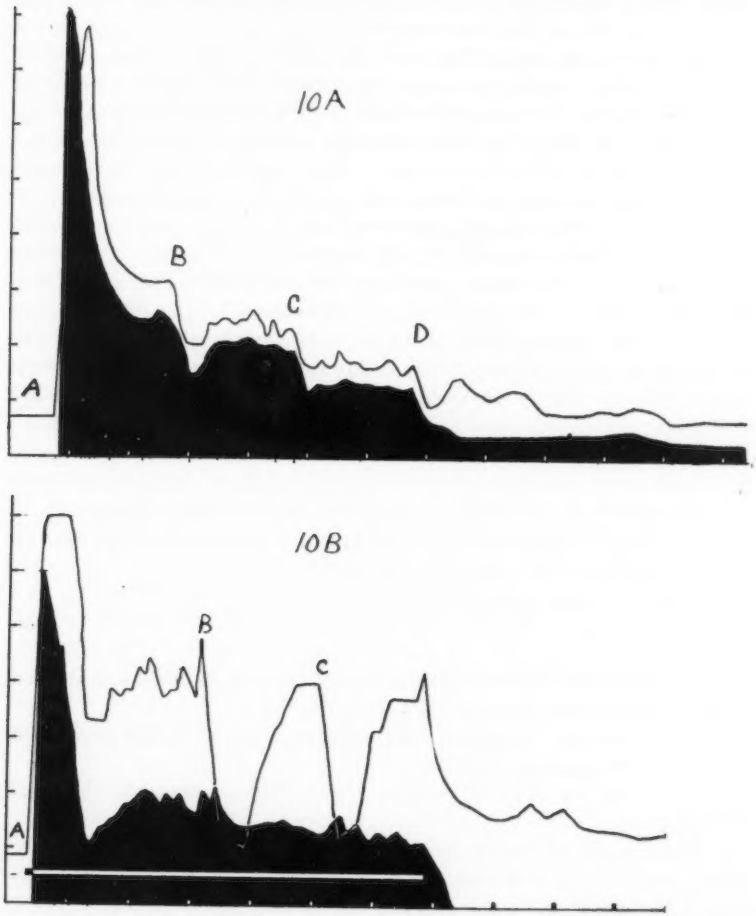

Fig. 10. Effect of suddenly reducing in steps the strength of a prolonged stimulation of the chorda tympani.

in rate of secretion, there was little if any change of rate. On the other hand the volume-flow of blood was enormously reduced—almost to the basal flow of blood. Though the strength of stimulation and rate

of secretion remained constant up to point *C*, the flow of blood remained reduced for a short period only, coming back again to the original superbasal flow. Another sudden reduction in the strength of stimulation at point *C* produced the same results.

It is exceedingly interesting that the reduced volume-flow of blood should suddenly accelerate though the strength of stimulation remained constant between the points of sudden diminution of strength of stimulation, to reach again the level obtaining between *A* and *B* where the stimulation is considerably stronger. From the fact that the volume-flow of blood diminished though the secretion remained constant, it would appear, assuming the presence of dilator fibers, that the strength of stimulation was reduced below the threshold of these fibers. Whether the subsequent acceleration of volume-flow of blood is due to an automatic lowering of the threshold of stimulation of the dilator fibers resulting from accumulation of metabolites or whether it is due to the direct action of the metabolities on the vessels, the results do not definitely indicate. They do show a lack of parallelism between metabolism and volume-flow and accepting the absence of constrictor fibers in the chorda tympani they point to the existence of dilator fibers. The value to be placed on these findings depends upon the significance we can attach to an isolated exception of this kind. It should be mentioned in this connection that although this result was obtained on only one animal it was obtained repeatedly.

SUMMARY

The dependence of tissue activity on volume-flow of blood was studied on the submaxillary gland of the dog—

a, by comparing the amount of secretion obtained during periods of normal and reduced flow of blood;

b, by noting the effect of decreased flow of blood upon the electrical deflections;

c, by studying the after-flow of blood following de-occlusion of the artery immediately following tissue activation. (An exaggerated after-flow of blood was used as an index to overstrain of the tissue).

Reduction of the volume-flow of blood during a short period of stimulation of the chorda tympani, from 10 to 30 seconds, did not decrease the amount of secretion.

The glandular processes, however, were affected by such procedure, for the electrical deflections were invariably altered.

Reduction of the volume-flow of blood during a period of more intense stimulation, but also of short duration, although it did not reduce the amount of secretion elicited, resulted in a more prolonged flow of blood as well as an altered electrical deflection.

With more prolonged stimulation of the chorda tympani the various glands responded differently to arterial occlusion. On some glands occlusion of the artery for a period of about 1 minute was without effect upon secretion, while in others a noticeable reduction in secretion occurred.

The temporary independence of tissue activity of volume-flow of blood as evidenced by secretion is probably apparent only and is due to the recovery of the gland between periods of stimulation and to the relative independence of the process of liberation of secretion on volume-flow of blood.

The dependence of tissue activity upon flow of blood is better shown by reducing the flow through a tissue which has already been activated for some minutes, that is, in a tissue in which recuperation and activity are going on hand in hand.

Such methods showed a very close dependence of tissue activity upon volume-flow of blood. The results substantiate the views previously published on the significance of hemorrhage and reduced flow of blood from other causes in the onset and sustentation of the condition of traumatic shock.

Prolonged stimulation of the chorda tympani usually produced fluctuations in secretion during the period of stimulation: first a rapid secretion followed by a decrease, then another acceleration giving way to a final decrease at the end of stimulation. Similar fluctuations were produced by a short period of stimulation lasting only a small part of the period of secretion. Whether the stimulation was long or short, the volume-flow of blood and the secretion ran parallel with each other. The significance of the findings is discussed.

The close parallelism between tissue activity and volume-flow of blood offered difficulties in demonstrating definitely the existence of dilator fibers.

One experiment showing a lack of this parallelism indicates the presence of dilator fibers in the chorda tympani.

Data are presented indicative of chemical regulation of blood flow.

Experiments are cited pointing to the absence of constrictor fibers in the chorda tympani.

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STUDIES ON THE SUBMAXILLARY GLAND

VII. On the Effects of Increased Salivary Pressure on the Electrical Deflections, the Volume-flow of Blood and the Secretion of the Submaxillary Gland of the Dog

ROBERT GESELL

From the Departments of Physiology of Washington University Medical School and the University of California

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In studying the electrical deflections of the submaxillary gland of the dog I noted that stimulation of the chorda tympani produced a greater and more prolonged after-flow of blood when the salivary duct was obstructed than when unobstructed (see figs. 4, 5 and 8). In so far as these observations aid in elucidating the mechanism of the control of volume-flow of blood they pertain to the problem of papers II, III, IV, VI and VIII of this series. The observations, however, will be considered from a broader point of view, namely, in their relation to the general problem of the physiology of the salivary glands.

The data will be discussed under the following heads:

1. Effects of increased salivary pressure upon the electrical deflections of the gland.
   a. Occlusion of the salivary duct during secretion elicited by the injection of pilocarpin.
   b. Occlusion of the duct synchronous with secretion elicited by the stimulation of the chorda tympani.
   c. Backward injection into the salivary duct of the resting gland.
2. Effects of increased salivary pressure upon the volume-flow of blood through the gland.
   a. Occlusion of the salivary duct during secretion elicited by the injection of pilocarpin.
   b. Occlusion of the duct synchronous with secretion elicited by the stimulation of the chorda tympani.
   c. Backward injection into the salivary duct of the resting gland.
3. Effect of increased salivary pressure upon secretion.
   a. Occlusion of the duct during secretion elicited by the injection of pilocarpin.
   b. Occlusion of the duct synchronous with secretion elicited by stimulation of the chorda tympani.

c. Backward injection into the salivary duct of the resting gland.

d. Effect of occlusion of the duct during secretion elicited by the stimulation of the chorda tympani upon secretion elicited by subsequent stimulation of the chorda tympani and vago-sympathetic.

e. Effect of backward injection in the resting gland upon subsequent secretion elicited by stimulation of the chorda tympani or vago-sympathetic before and after atropinization.

1. EFFECTS OF INCREASED SALIVARY PRESSURE UPON THE ELECTRICAL DEFLECTIONS OF THE GLAND

a. Occlusion of the duct during secretion elicited by the injection of pilocarpin. When the submaxillary gland is activated by the injection of pilocarpin a definite electrical deflection occurs and when the secretion approaches a constant rate the two electrodes tend again to assume a constant difference of potential as is evidenced by the horizontal direction of the recorded electrical deflection. If the salivary duct is now occluded a typical disturbance of this equilibrium occurs which is shown in figure 1, *A*, *B*, *C*, *D* and *E*. The first effect of occlusion was an upward deflection which gave way in a few seconds to a downward deflection. De-occlusion produced the reverse effect. The downward deflection accompanying occlusion was suddenly accelerated and changed as suddenly into an upward deflection. The contour of the deflection differed considerably from time to time and from animal to animal as is obvious from figure 1, *A*, *B*, *C*, *D* and *E*, yet the four phases were present in all. The electrical deflection elicited by occlusion and de-occlusion of the duct may therefore be looked upon as more or less accurately indicating the sequence of certain glandular processes set up by these procedures.

b. Occlusion of the salivary duct synchronous with secretion elicited by the stimulation of the chorda tympani. If the chorda tympani is stimulated at regular intervals with stimuli of equal strength and duration equal amounts of saliva may be elicited and electrical deflections of the same contour may be obtained, provided, the period of rest intervening between stimulations is of such duration as to prevent the augmenting effect of previous excitation. When such constant results were obtained it was found that occlusion of the salivary duct along with stimulation of the chorda tympani produced a definite change in the electrical deflection as is seen in figures 2 and 3. These figures show five sets of observations—2, *A*, *B* and *C*, and 3, *A* and *B*. In any single set of observations the chorda tympani was stimulated at

equal intervals of time with equal strength of stimulation and the duct occluded equal lengths of time. But in the different sets of observations the periods and strength of stimulation, the periods of rest and of occlusion of the duct were variable. This variability must in part

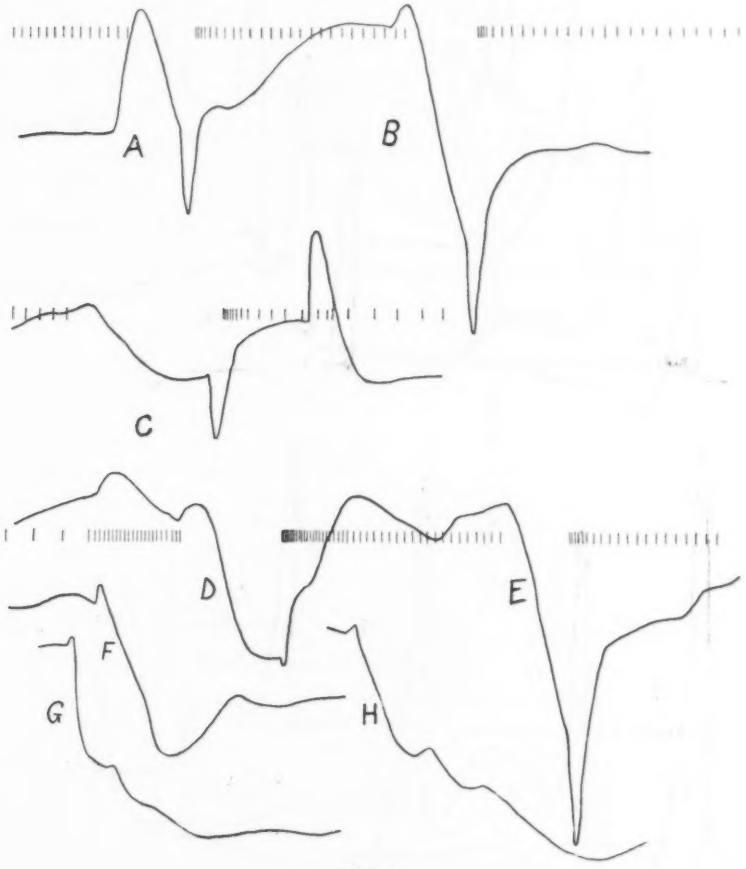

Fig. 1

account for the differences of the deflections. Bearing the variability of the conditions of the experiments in mind it is quite remarkable that the changes in contour produced by occlusion of the duct should be as uniform as they are.

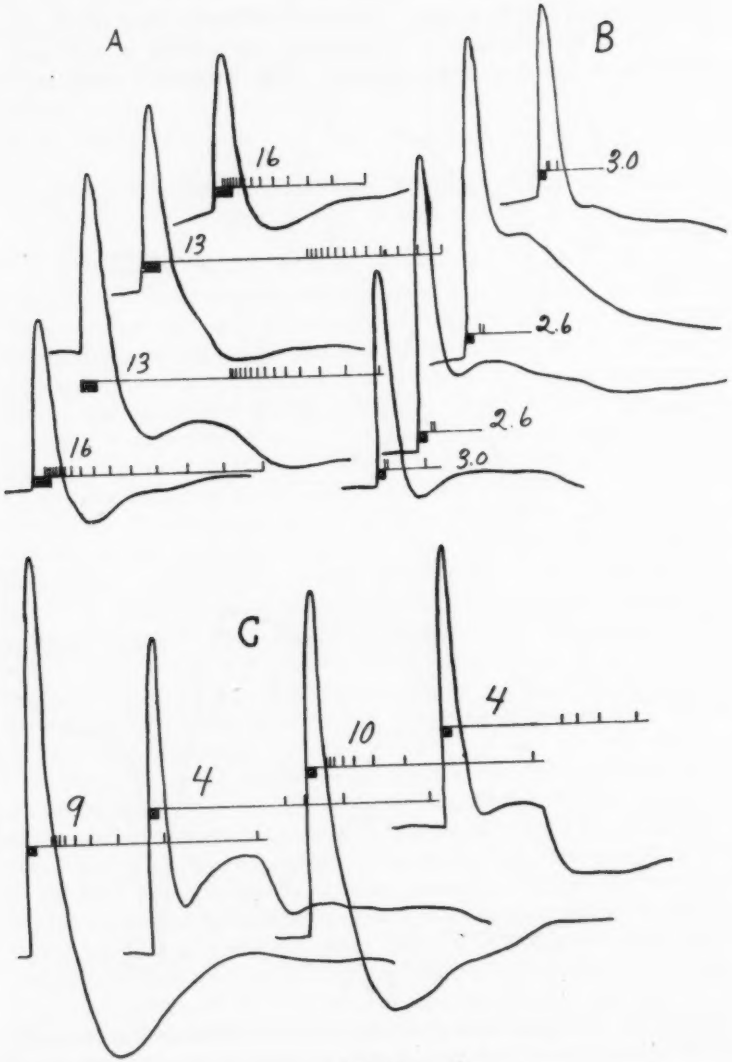

Fig. 2

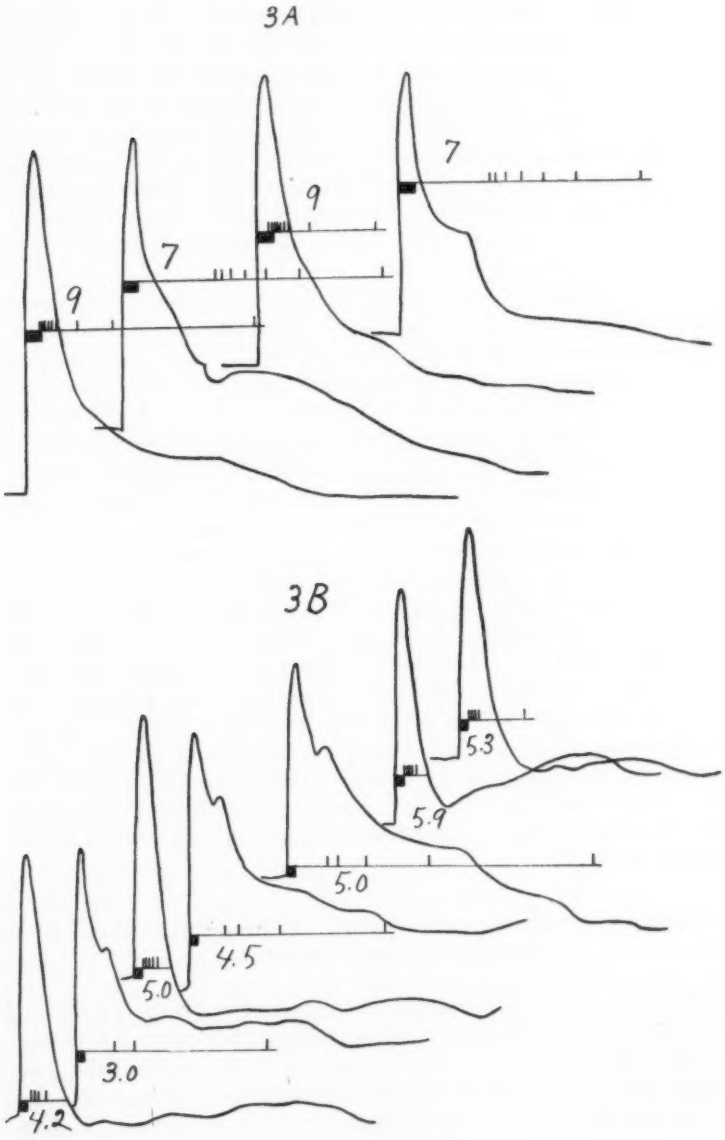

Fig. 3

Prolongation of the electrical disturbance is a point more or less common to the observations in which occlusion of the duct occurred. This prolongation is associated with a prolongation of the normal period of secretion. Other characteristics common to many of the modified deflections are the notch and the elevation of the base line. To be sure there are some dissimilarities in the deflections of different groups of observations, yet in any single set of observations repeated occlusion produced changes in the deflections so nearly alike that here again the deflections seem to indicate with considerable exactness the sequence of glandular processes.

In some instances de-occlusion of the duct produced a downward deflection similar to that associated with de-occlusion during secretion from the injection of pilocarpin. But it is obvious that the deflection as a whole showing the effects of occlusion and de-occlusion cannot be compared to advantage with the deflections obtained by occlusion and de-occlusion during secretion from the injection of pilocarpin, for in one instance the deflection is a result of a change in secretion already in progress and in the other it is a resultant of the activation of the resting gland and the obstruction of the secretion formed.

c. Backward injection into the salivary duct of the resting gland. The backward injection of saline or gum-saline produced deflections comparable to those elicited by occlusion of the duct during active secretion elicited by the injection of pilocarpin. Such deflections are shown in figure 1, *F*, *G* and *H*. The injection produced an upward deflection followed by a downward deflection. Cessation of the backward injection was associated with a downward deflection followed by an upward deflection. The general contour of the deflection, to be sure, differs from that produced by occlusion during active secretion, yet the four phases are present. It is of interest to note here that atropinization apparently does not influence the effects of backward injection upon electrical deflections.

2. EFFECTS OF INCREASED SALIVARY PRESSURE UPON THE VOLUME-FLOW OF BLOOD THROUGH THE GLAND

a. Occlusion of the salivary duct during secretion elicited by the injection of pilocarpin. Occlusion of the salivary duct during secretion resulting from the injection of pilocarpin as a rule retarded the flow of blood during the period of occlusion, as is shown in figure 5, *A*. On de-occlusion the flow accelerated again to reach or surpass the flow

preceding occlusion. The de-occlusion flow of blood in figure 5, *A*, only approximated the pre-occlusion flow, but in consideration of the fact that the volume-flow of blood as a rule is proportional to the activity of the gland and of the fact that the de-occlusion secretion was considerably slower than the pre-occlusion secretion, the results suggest that even in this observation occlusion in reality produced an accelerated after-flow of blood.

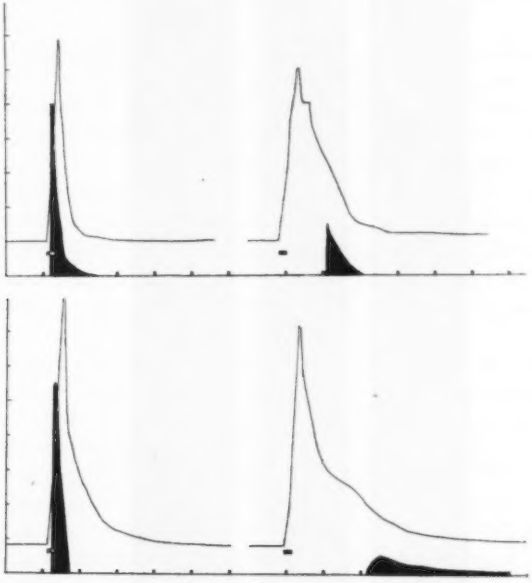

Fig. 4

b. Occlusion of the duct synchronous with secretion elicited by stimulation of the chorda tympani. The above results are substantiated by the universally accelerated after-flow of blood noted on de-occlusion of the duct after occlusion synchronous with secretion elicited by stimulation of the chorda tympani (see figs. 4 and 6). A comparison of record *B* of figure 6, in which occlusion of the duct occurs, with records *A* and *C*, in which there was no occlusion of the duct, shows the accelerated flow of blood. The results of two such experiments are plotted in figure 4 in which secretion (solid black) and volume-flow of blood (single line) are plotted on the ordinates against time in minutes

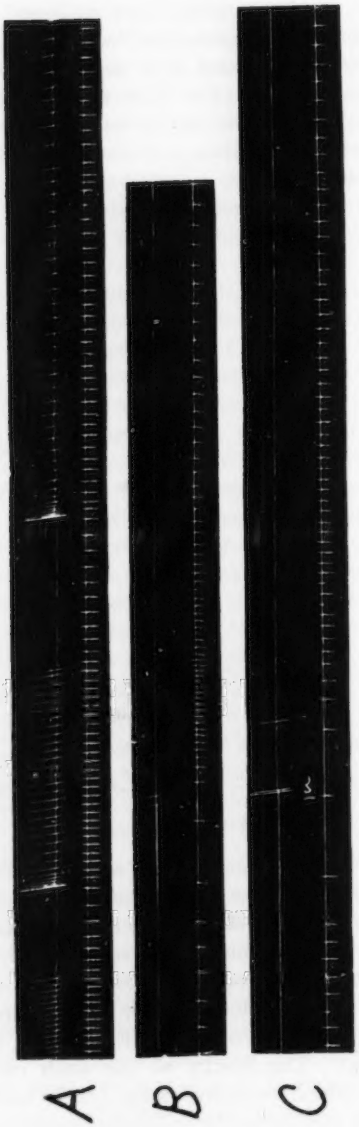

Fig. 5

on the abscissas. The duration of stimulation of the chorda tympani is marked by the rectangle near the abscissas at the beginning of the record. The extra-flow of blood elicited when the duct was occluded greatly exceeded the extra-flow occurring when the duct was not occluded. Though the two experiments represented in this figure show little reduction of the flow of blood during the occlusion of the duct, such slowing not infrequently occurred, in agreement with the results produced by occlusion of the duct during secretion elicited by the injection of pilocarpin.

c. Backward injection into the salivary duct of the resting gland. Backward injection into the salivary duct of the resting gland produced comparable results to those obtained by increasing the salivary pressure in the two ways discussed above (see fig. 5, *B* and *C*). During the period of increased salivary pressure the flow of blood was greatly reduced. On cessation of injection, which is indicated by the salivary record, the volume-flow of blood accelerated to remain accelerated for several minutes above the basal-flow of blood preceding injection.

3. EFFECT OF INCREASED SALIVARY PRESSURE UPON SECRETION

a. Occlusion of the duct during secretion elicited by the injection of pilocarpin. De-occlusion of the salivary duct after occlusion during secretion elicited by the injection of pilocarpin was followed by a momentary rapid acceleration of secretion which within a few seconds usually gave way to a rate of secretion below that obtaining before occlusion (see fig. 5, *A*). How much of the momentary accelerated secretion was due to the emptying of distended ducts, to the passage of saliva which escaped into the tissue spaces back into the ducts, to the liberation of secretion accumulated in the cells themselves due to failure to overcome the increased salivary pressure in the ducts, the experiments do not show. The retarded secretion following de-occlusion suggests some form of tissue damage resulting, possibly, from backward filtration. The effect which occlusion of the duct has upon the rate and amount of secretion following de-occlusion depends largely upon the duration of occlusion, the rate of the pre-occlusion secretion and the variability common to the glands themselves. Some saliva is always unaccounted for in the compensatory secretion.

b. Occlusion of the duct synchronous with secretion elicited by stimulation of the chorda tympani. Occlusion of the salivary duct during stimulation of the chorda tympani followed by subsequent de-occlusion

produced variable results, depending again upon the rate of secretion elicited by stimulation, the duration of occlusion of the duct and the peculiar reaction of the gland itself. Various results from several animals are shown in figures 2 and 3. The records in each case are arranged in their proper sequence. Figure 2, *A* and *B*, which is compiled from two different animals, shows the effects of prolonged occlusion of a copious secretion and a momentary occlusion of a scant secretion. In figure 2, *A* the chorda tympani was stimulated at regular intervals of 4 minutes with a constant strength of stimulation lasting 16 seconds. With the duct unobstructed each stimulation elicited 16 drops of secretion as is shown in the first record of that series. In the following two records where the duct was occluded for 2.5 minutes 13 drops of saliva were secreted following de-occlusion, that is, only 3 drops of secretion were lost. In the final control when the duct was unoccluded 16 drops were again elicited. In the following record, 2, *B*, a scant secretion of only 3 drops of secretion was elicited by a relatively weak stimulus lasting 5 seconds. The duct was occluded only 10 seconds. It will be noted that occlusion of such scant secretion for only 10 seconds resulting in a loss of about 13 per cent of the secretion and producing a definite change in the electrical deflection, stands in striking contrast to the loss of only 3 drops out of a larger total of 16 drops of secretion obstructed for 2.5 minutes. The results in figure 2, *B* indicate that the storage capacity of the ducts of a gland weighing approximately 7 gm. may be little over 2 drops and that if an amount greater than 2 drops is obstructed, enough back pressure may be developed to interfere with further secretion. If that is true, where were the 13 drops of the experiment represented in 2, *A* stored?

c. Backward injection into the salivary duct of the resting gland. An index to the capacity of the ducts as it pertains to this problem may be indicated by the after-flow of secretion following backward injection of gum-saline in the resting gland. When a small amount of fluid was injected and then retained by occlusion of the duct, subsequent de-occlusion within 40 to 80 seconds was usually followed by several drops of after-flow. Not infrequently there was no after-flow whatever; but the usual flow of 2 to 4 drops suggests again that the ducts may accommodate, for a short time at least, approximately 3 drops of saliva.

d. Effect of occlusion of the duct during secretion elicited by the stimulation of the chorda tympani upon secretion elicited by subsequent stimulation of the chorda tympani and the vago sympathetic. The effects of

occlusion of the duct during secretion elicited by the stimulation of the chorda tympani upon secretion elicited by subsequent stimulation of the chorda tympani and vago sympathetic are shown in figure 6. This figure gives the results of two sets of observations, *A-D* and *W-Z*. The record of the first set shows secretion and volume-flow of blood; the record of the second set electrical deflections as well. The chorda tympani was stimulated at regular intervals with stimuli of equal strength and duration. Equal amounts of secretion were elicited with each stimulation under normal conditions. During one such period of stimulation the salivary duct was occluded and the effect upon the subsequent stimulation noted. In record *A* a total of 12 drops of saliva was secreted. Since 3 of these drops were secreted slowly after the cessation of stimulation, only 7 drops were rapidly secreted during the period of stimulation. In record *B* the duct was occluded during stimulation of the chorda tympani and several minutes after stimulation the duct was de-occluded whereupon only one drop of saliva fell from the cannula. Next, record *C*, the chorda tympani was stimulated with the duct de-occluded; 13 drops of saliva were secreted, of which 11 drops appeared within the 10-second interval of stimulation. In record *D*, the final control, 6 drops of saliva were secreted rapidly. It follows from these results that occlusion of the duct during secretion increased the subsequent secretion by 4 or 5 drops.

The augmenting effect of occlusion upon secretion is shown still better in observations *W*, *X*, *Y* and *Z*, due to the fact that after-secretion was absent. In record *W* stimulation of the chorda tympani for a period of 10 seconds elicited 4 drops of saliva. In record *X* the duct was occluded during stimulation. A rapid flow of blood occurred. There was no flow of saliva on de-occlusion of the duct. In the following record, *Y*, stimulation elicited 8 drops of saliva or double the amount in record *W*. In the final control approximately 3 drops were again secreted. Note the electrical deflections.

The effects of occlusion of the salivary duct during secretion elicited by stimulation of the chorda tympani on subsequent secretion elicited by stimulation of the vago sympathetic appear to be identical with those just described (see fig. 7). The lower broken line on which time is marked in seconds indicates when the chorda tympani was stimulated and the upper line when the vago-sympathetic was stimulated. The results of alternate stimulation of the chorda tympani and vago-sympathetic without occlusion of the duct are shown in the upper tracing; with occlusion, in the lower tracings. Without previous occlusion of

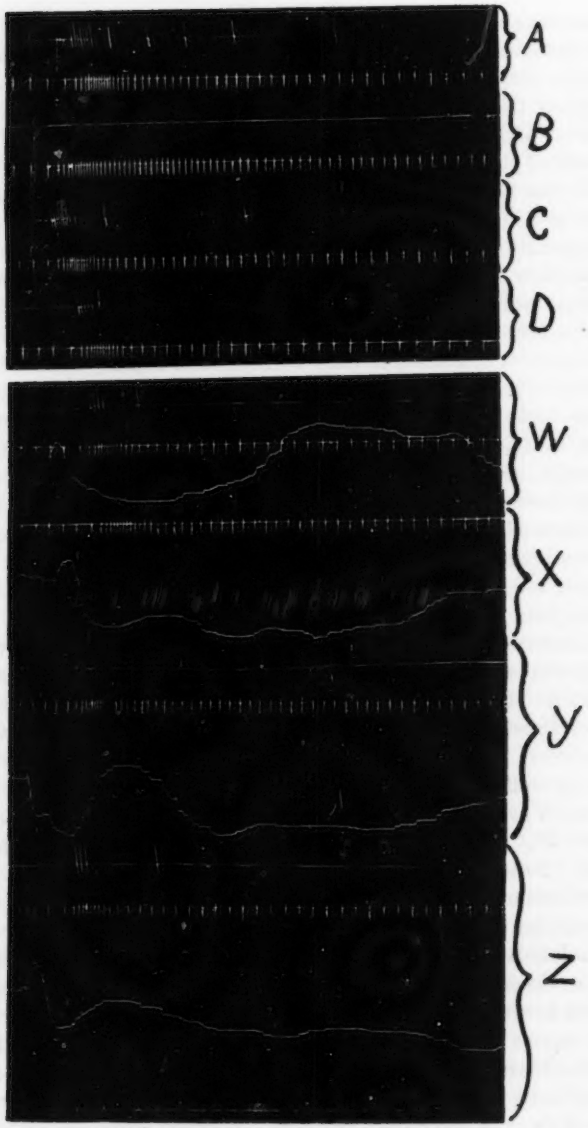

Fig. 6

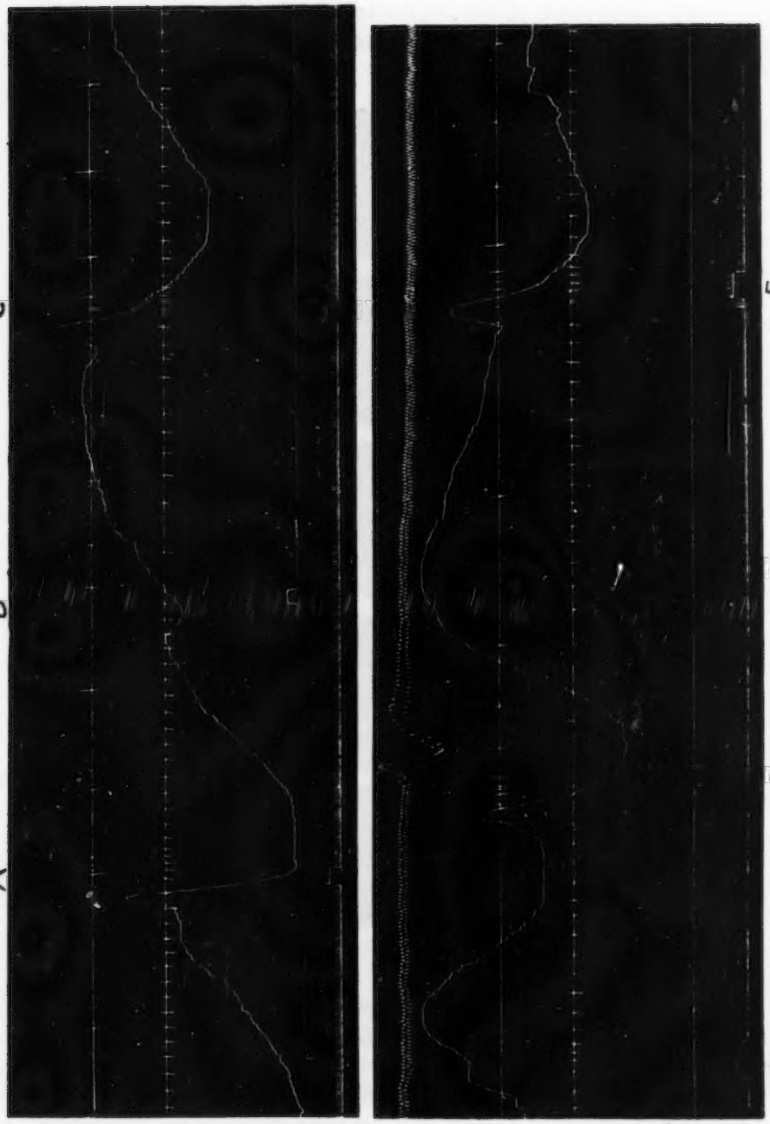

Fig. 7

the duct stimulation of the vago-sympathetic elicited a slow scanty secretion of only 3 drops, but with previous occlusion a secretion of 8 drops.

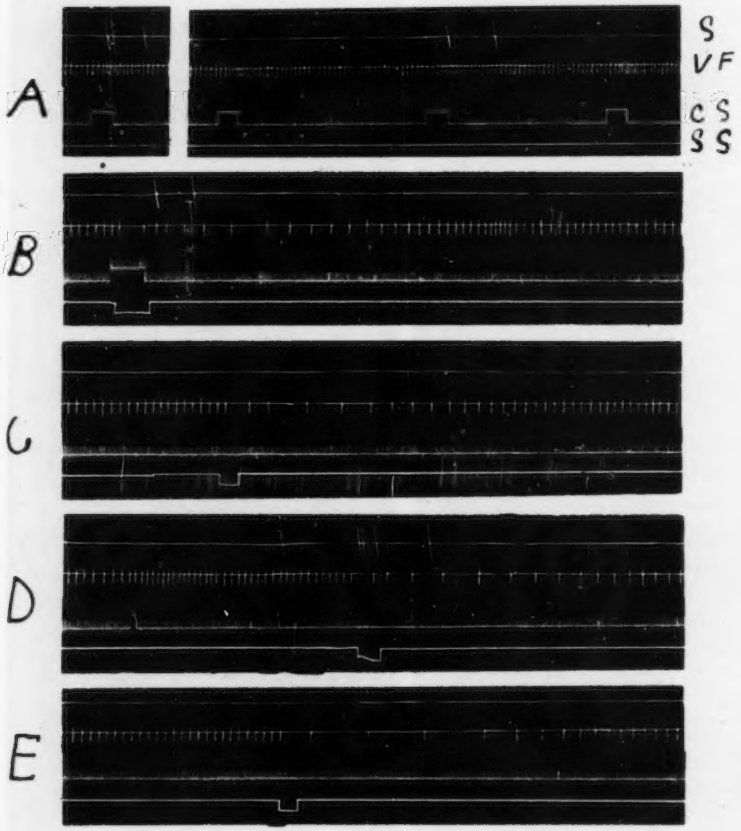

Fig. 8

e. Effect of backward injection in the resting gland upon subsequent secretion elicited by the stimulation of the chorda tympani or vago-sympathetic before and after atropinization. When equal amounts of saliva were elicited at regular intervals by equal stimulation and gum-saline was then momentarily injected into the duct, it was found that stimu-

lation of the chorda tympani 1 to 5 minutes following the injection elicited a greater amount of secretion and that the latent period of secretion was reduced by several seconds. The same reduction in latent period was noted when stimulation of the chorda tympani was preceded by occlusion of the duct during secretion elicited by stimulation of the chorda tympani.

The experiment represented in figure 8 shows the effect of atropin upon the results described above. In record *A*, the first stimulation of the chorda tympani elicited 3 drops of saliva. Following that stimulation 1 mgm. of atropin was injected intravenously. This effectively paralyzed the chorda tympani for the prevailing stimulus, as is shown by the absence of secretion with the second stimulation. Gum-saline was then injected backwards through the duct into the gland and the duct de-occluded within a minute. The next stimulation elicited 2 drops of secretion though the subsequent stimulation was ineffective. In record *B*, a continuation of the experiment, the chorda tympani and vago-sympathetic were simultaneously stimulated producing 2 drops of secretion. The accelerated volume-flow of blood shortly following was a result of the injection of 11 mgm. of atropin. Stimulation of the vago-sympathetic following that injection in record *C* was ineffective so far as secretion is concerned, but reduced the flow of blood. In record *D*, gum-saline was injected into the duct between the breaks on the time record. One drop left the cannula on de-occlusion. Subsequent stimulation of the vago-sympathetic yielded 4 drops and final stimulation none. Similar results have been obtained after the intravenous injection of 350 mgm. of atropin.

DISCUSSION

From the work of Langley we know that during rest some of the constituents of salivary secretion are elaborated and stored ready for subsequent secretion. It appears that even after the glandular cells are well stocked with zymogen granules, secretion may yet occur in two definite stages, final elaboration followed by ultimate liberation. Various observations point in that direction, e.g., the observation of Langley on the augmenting effect of stimulation of the chorda tympani upon the secretion elicited by subsequent stimulation of the sympathetic fibers. Stimulation of the chorda tympani too weak to elicit secretion may produce a definite electrical deflection. In the preceding paper it was noted that a gland subjected to a subnormal flow of blood failed

to secrete during the period of stimulation of the chorda tympani, whereas it secreted 20 to 30 seconds after cessation of stimulation when the flow of blood through the gland was suddenly accelerated. The observation in the present paper, that a gland may continue to secrete for a period of 4 or 5 minutes after the cessation of stimulation, as is shown in the de-occlusion experiments, is likewise of significance; as is also the fact that injection of atropin in amounts sufficient under usual experimental conditions to paralyze the secretory endings of the chorda tympani, fails to prevent a discharge from the gland on stimulation of the chorda tympani after a previous injection of gum-saline into the duct of the gland.

Unfortunately we do not understand the reaction of the gland to increased salivary pressure. The fact that a storage of more than 2 drops of saliva in the ducts embarrasses the gland and that 13 drops of saliva may be secreted after the duct is occluded for a period of 2.5 minutes indicates that at least not all of the after-secretion following de-occlusion is due to an expression of saliva contained within the distended ducts. The slowness of the secretion would also speak against this. Yet we have some evidence pointing to the contractility of the salivary ducts; for example, when the gland is secreting at an even rate as a result of an injection of pilocarpin, a short stimulation of the chorda tympani results in a momentary acceleration of secretion followed by a compensatory slowing which in turn gives way to a rate approaching the initial rate.

We know that when secretion occurs against a high resistance much of the saliva is filtered backward into the interspaces of the gland. The extreme slowness of secretion in certain instances following de-occlusion of the duct suggests the passage of this saliva back into the ducts. So far as I know we have no evidence that an increased salivary pressure results in filtration of saliva back into the secreting cells themselves or prevents the liberation of saliva which is already in these cells. Since "secretion" may be elicited following backward injection after atropinization sufficient to paralyze secretion, there is the possibility that under more normal conditions fluid is filtered backward into the cells, this fluid being later liberated. A part of the excess fluid within the cells may be liberated by a passive mechanism on the reduction of the salivary pressure accompanying de-occlusion, but the passive mechanism may not be sufficient to liberate all of the excess fluid or secretion and this may then be actively liberated upon stimulation of the chorda tympani. It seems not improbable that this active libera-

tion may be the result of active contraction of certain constituents of the secreting cells themselves. This problem needs further investigation and will be reported upon later. The results so far indicate only that atropin in certain stages of atropinization paralyzes primarily the function of elaboration of secretion leaving the function of liberation more or less intact.

As to the nature of the changes in volume-flow of blood produced by increased salivary pressure we can say that they are not of a central reflex origin if all the vasomotor nerves to the gland run in the chorda tympani and vago-sympathetic, for these changes in flow occurred after section of both nerves. The decreased flow obtaining during the period of increased pressure may well be a mechanical effect of compression of the capillaries and venules, for it obtains during stimulation of the chorda tympani when the factors normally producing dilatation are at work, as well as when the gland is at rest. On de-occlusion of the duct the compressing pressure vanishes and the flow of blood not only returns to normal but greatly exceeds the normal. When this accelerated flow was first noticed with occlusion accompanying stimulation of the chorda tympani it was thought that possibly more energy was required for secretion against a high pressure and that the accelerated flow of blood supplied this needed energy. The accelerated flow resulting from backward injection into the duct of the resting gland is contradictory to this view. The accelerated flow does not seem to be due to a specific chemical irritation of the saliva itself for it occurs after injection of inert solution, such as saline and gum-saline. Whether a backward filtration into the secreting cells would bring about increased volume flow of blood we do not know. The only suggestion we have at present to account for the accelerated flow is the tissue damage resulting from backward filtration into the tissue spaces and possibly into the cells themselves.

SUMMARY

Occlusion of the duct of the submaxillary gland of the dog during secretion elicited by the injection of pilocarpin produced characteristic electrical deflections.

Injection of gum-saline into the duct produced somewhat comparable deflections.

Occlusion of the duct along with stimulation of the chorda tympani modified in a more or less typical way the usual electrical deflection obtained by stimulation when the duct was not occluded.

Occlusion of the salivary duct during secretion elicited by the injection of pilocarpin retarded the flow of blood. The after-flow of blood was at times accelerated.

Backward injection of gum-saline into the salivary duct of the resting gland retarded the flow of blood during the period of increased pressure. Release of that pressure resulted in an accelerated flow of blood at times lasting many minutes.

Occlusion of the duct during secretion elicited by stimulation of the chorda tympani frequently retarded the flow of blood. De-occlusion was followed by a markedly accelerated after-flow of blood.

De-occlusion of the salivary duct during secretion elicited by the injection of pilocarpin was followed by a short period of accelerated secretion. This accelerated secretion never compensated fully the absence of secretion during the period of occlusion.

De-occlusion of the duct several minutes subsequent to injection of gum-saline into the duct was followed by an after-flow from the duct of 0 to 4 drops.

Occlusion of the salivary duct along with stimulation of the chorda tympani was followed upon de-occlusion by a secretion less in amount than that normally elicited with the duct unoccluded. The amount of saliva unaccounted for varied considerably with the animal, the duration of occlusion and the amount of secretion obstructed.

De-occlusion of the duct after obstructing for several minutes a temporary secretion elicited by stimulation of the chorda tympani may be followed by a slow secretion lasting 4 to 5 minutes.

Occlusion of the duct during secretion elicited by the stimulation of the chorda tympani increased the amount of saliva elicited by subsequent stimulation of the chorda tympani or vago-sympathetic. The latent period of secretion was also reduced by several seconds.

Backward injection of gum-saline into the resting gland increased the amount of secretion el cited by subsequent stimulation of the chorda tympani or vago-sympathetic and reduced the latent period of secretion.

Injection of atropin sufficient to check secretion elicited by the stimulation of the chorda tympani or vago-sympathetic may fail to prevent secretion with similar stimulation after backward injection into the salivary duct.

If all the nerve fibers reaching the submaxillary gland run in the chorda lingual and vago-sympathetic the changes in volume-flow of blood resulting from increased salivary pressure are not of a central reflex origin.

The decreased volume-flow of blood during the period of increased salivary pressure is probably due to mechanical occlusion of the capillaries or venules.

It is suggested that the accelerated flow of blood following de-occlusion is a result of tissue damage from backward filtration into the tissue spaces, and possibly into the cells themselves.

The effects of occlusion of the duct upon secretion elicited by stimulation of the chorda tympani suggests that secretion may occur in two definite stages. The effects of atropin on augmented secretion following backward injection of gum-saline into the duct suggests the same inference.

STUDIES ON THE SUBMAXILLARY GLAND

VIII. On the Effects of Atropin upon Volume-flow of Blood, Electrical Deflections and Oxidations of the Submaxillary Gland

ROBERT GESELL

From the Departments of Physiology of the Washington University Medical School, St. Louis, and the University of California, Berkeley, California

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When the chorda tympani is stimulated definite effects are produced in the submaxillary gland among which are: secretion of saliva, an augmented flow of blood, a change in the electrical condition of the gland and an accelerated oxidation. This paper considers briefly some of the effects of the intravenous injection of atropin upon these processes.

The work is a continuation of the study of electrical deflections of the submaxillary gland and has a bearing upon the problems of vasomotor control. In paper II of this series (1) the nature of the electrical deflections elicited by stimulation of the chorda tympani was discussed. It was pointed out that such factors as the type of lead, the strength and duration of stimulation, the period of rest, etc., all influence the kind of deflection obtained. It is essential then to keep these factors in mind in connection with the effects which atropin produces upon electrical deflections elicited by stimulation of the chorda tympani.

We know from the work of others the effect which atropin has upon secretion of saliva. These effects, when noted in this work will, therefore, be discussed only in their connection with the changes in volume-flow of blood, the electrical condition of the gland and oxidations.

Atropin influences the electrical response of the gland profoundly as was shown by Bayliss and Bradford (2). I have obtained like results.

Figure 1 shows the effect of the injection of 1.5 mgm. of atropin. Record *A*, shows the effects of stimulation of the chorda tympani with both vago-sympathetics intact before the injection of atropin. Record *D*, shows the effects of stimulation after double vago-section and atropinization. Records *B* and *C*, show the effects of section of the left and right vago-sympathetics respectively. Atropinization abolished

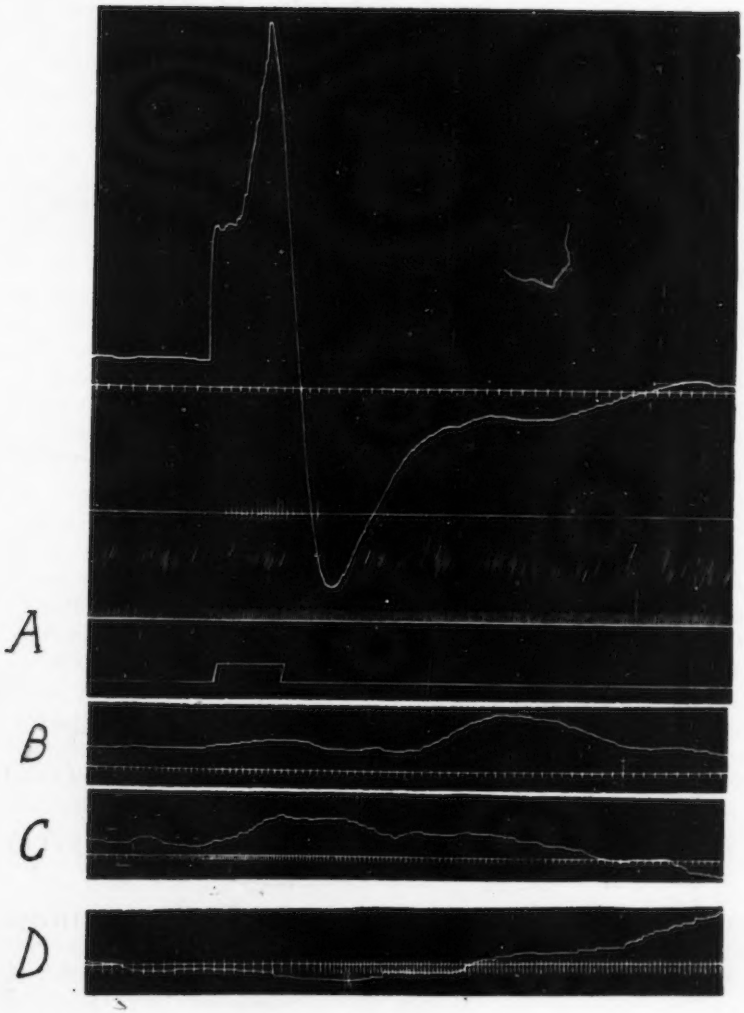

Fig. 1

both the secretion and the electrical deflection elicited by stimulation of the chorda tympani, and from records *B* and *C*, it is apparent that

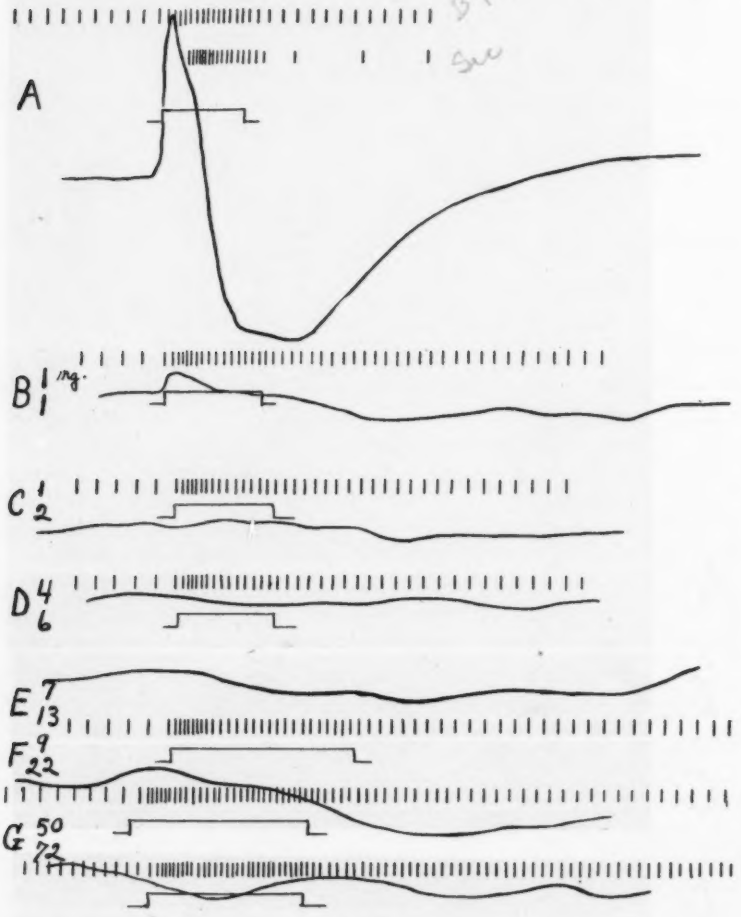

Fig. 2

volume-flow of blood per se was of little if any significance in the development of electrical changes. In record *A*, where the volume-flow of blood was least affected the largest electrical deflection occurred, and

in records *C* and *D*, where the flow was enormously accelerated, both passively and actively, there was practically no change in the electrical deflection.

Figure 2 shows the effects of repeated injections of small amounts of atropin, each injection occurring several minutes before stimulation of the chorda tympani. The amounts injected are noted on the record —the upper figure represents the amount injected prior to stimulation and the lower figure the total amount injected at that moment. In record *A*, before the injection of any atropin, stimulation of the chorda tympani elicited a copious secretion, a copious flow of blood and a large electrical deflection. In record *B*, after the injection of 1 mgm. of atropin, similar stimulation elicited approximately the same flow of blood but the secretion was reduced to only ½ drop and the electrical deflection was nearly abolished. The injection of another milligram of atropin before record *C*, although exerting no further visible effect upon the volume-flow of blood, paralyzed secretion and abolished the electrical deflection. The subsequent records followed the injections of 4, 7, 9 and 50 mgm. of atropin respectively. The electrical response of the gland continued to be absent, at least the deflections which occurred during the period of stimulation of the chorda tympani were no greater than those which occurred during the periods of rest. At times the accelerated flow of blood resulting from stimulation of the chorda tympani was reduced by the injection of atropin as is apparent from a careful comparison of records *A* and *B* of figure 3. It is of interest to note in connection with figure 2 that although the accelerated superbasal flow of blood was slightly reduced, the after-flow was greatly prolonged so that the sum total of the effects was a greater superbasal flow for equal stimulation after the administration of atropin. Note that in the lower records the basal flow of blood is considerably accelerated. The acceleration was not due to a rise of blood pressure. It occurred after section of the chorda tympani and the vago-sympathetic and therefore was peripheral in origin.

Figures 3, 4 and 5 show results of other experiments. In figure 3, the administration of 0.08 mgm. of atropin nearly paralyzed secretion for the prevailing stimulus, only 1 drop was elicited as compared with 6 drops before atropinization. It likewise reduced the superbasal flow of blood. The electrical deflection was still very distinct. In figure 4, taken from a different animal, a similar injection abolished secretion entirely. The electrical deflection was still prominent and the superbasal flow of blood during the period of stimulation was hardly affected,

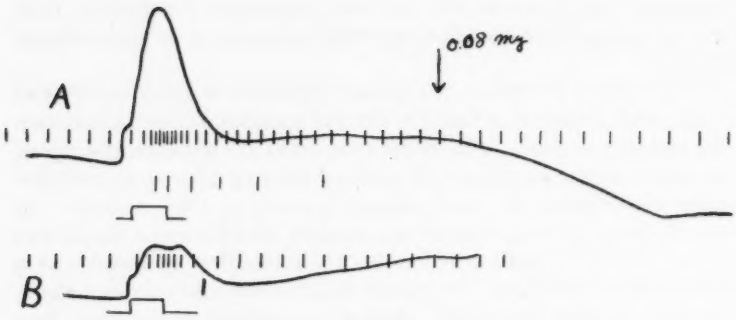

Fig. 3

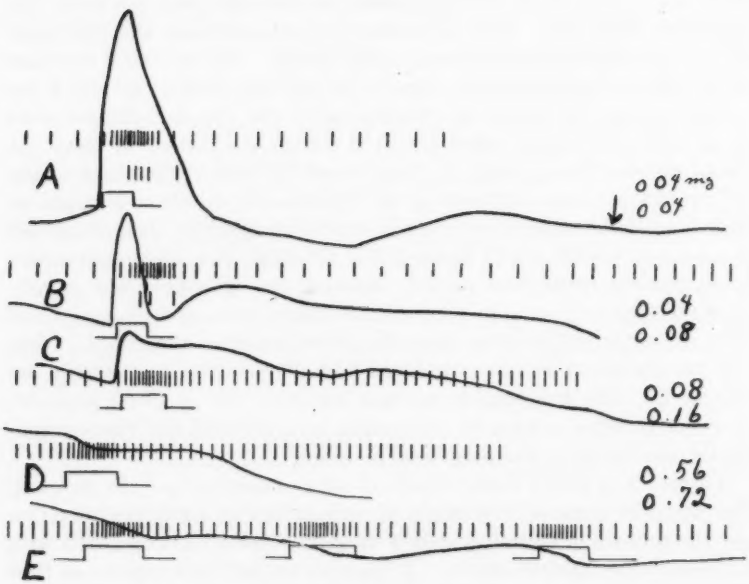

Fig. 4

Fig. 5

but the prolongation of the superbasal flow of blood occurred again. Further injections abolished the upward deflection, but it will be noted that stimulation of the chorda tympani then produced a very small downward deflection. Though atropin usually abolished the electrical deflection this reversal was not an uncommon occurrence; but as a rule it was of small magnitude and occurred even after large injections of 30 or more milligrams of atropin.

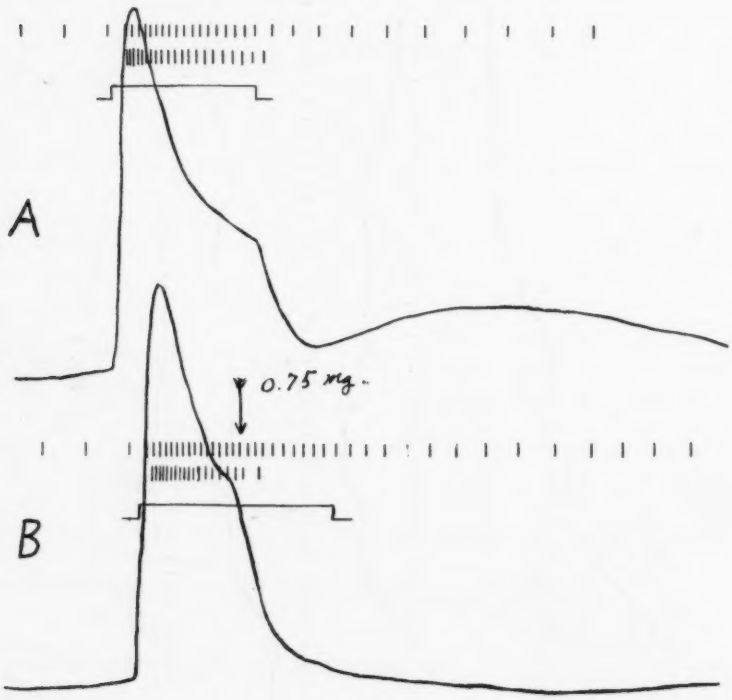

Fig. 6

If the injection of atropin prevents the development of an electrical deflection when the chorda tympani is stimulated, it might be logical to expect the injection of atropin to abolish an electrical deflection which is already in progress as a result of a continuous stimulation of the chorda tympani. Figures 6 and 7 show the results obtained on this point. In record *A*, figure 6, the chorda was stimulated before atropini-

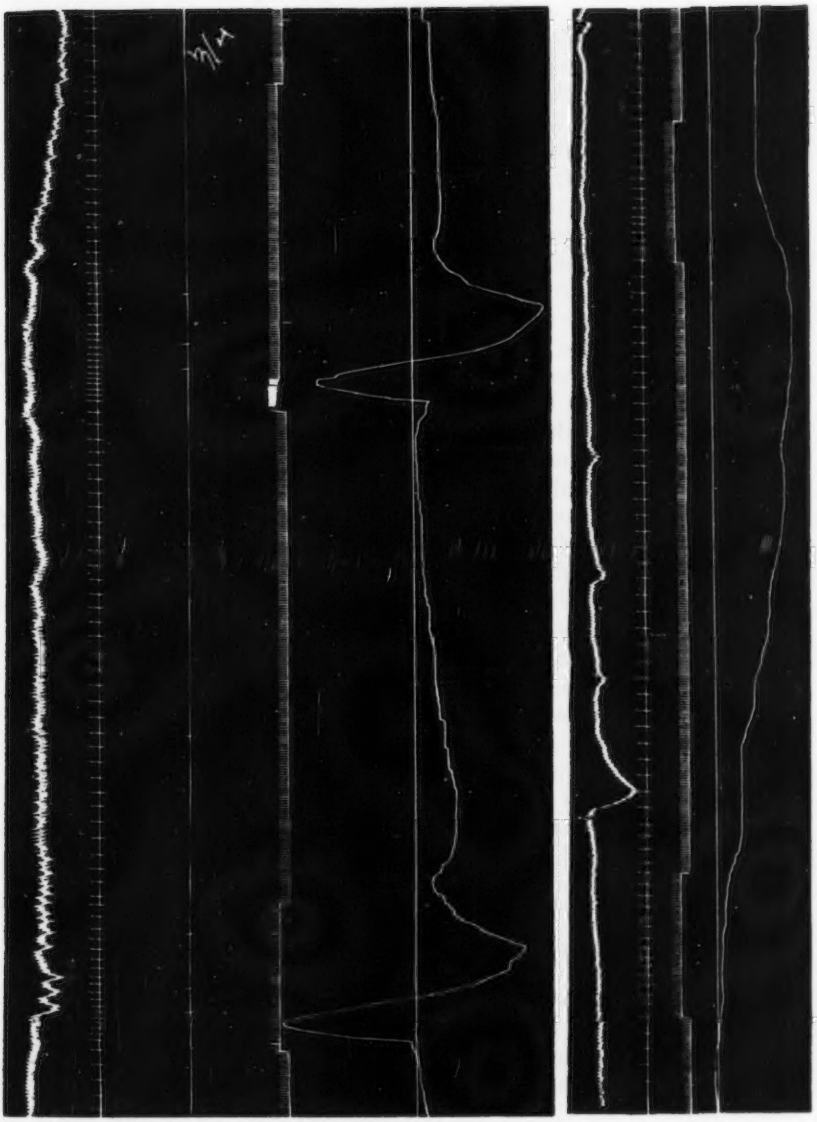

FIG. 7

zation. At the cessation of stimulation a sharp downward deflection occurred. In record *B*, atropin was injected during the period of stimulation. When the atropin reached the gland the same sharp downward deflection occurred, although the stimulation of the chorda tympani continued. In figure 7, record *B*, where the atropin reached the gland at relatively the same time interval as cessation of stimulation in record *A*, the electrical deflections were nearly identical. From the electrical deflections one might conclude, neglecting the volume-flow of blood, that atropinization during stimulation of the chorda tympani and cessation of stimulation call forth the same effects.

It is obvious that we know too little about electrical phenomena in living tissues to arrive at such a definite conclusion, yet I have tried in an indirect way to put this conclusion to the test. It should be possible to grade the strength of stimulation, or perhaps more correctly stated, the end effect of stimulation, in two ways—mechanically by regulating the strength of shock delivered by the induction coil, and physiologically by reducing the effectiveness of stimulation of constant strength by the injection of graded doses of atropin. A comparison of the electrical, secretory and vasomotor response of the gland with these two methods of gradation of stimulation seemed worth while attempting. The results obtained on two animals are shown in figures 8 and 9. The chorda tympani was stimulated at regular intervals with stimuli of equal duration. In the first observation in both experiments the stimulation was too weak to elicit visible secretion. The strength of stimulation was then increased with each observation up to an arbitrary maximum. That maximum was then kept constant for the remaining series of observations, but prior to each stimulation small amounts of atropin were injected, as small as 0.05 mgm. Each increase in strength of stimulation elicited an increase in the amount of secretion and a decided change in the electrical deflection. After the maximum strength of stimulation was reached each injection reduced the amount of secretion and elicited just as decided changes in the electrical deflection. The records show the results obtained and hardly need a lengthy discussion. To be sure, the corresponding deflections obtained with increasing and decreasing activation as indicated by the secretion are not superimposable, yet if we carefully compare the tracings of figure 8 keeping three factors in mind—the magnitude of the upward deflection, the reversal of the deflection to a downward deflection, and roughly, the general contour—definite similarities in the deflections of the two series appear. Considering the

fact that identical deflections can be obtained only when many factors remain perfectly constant and that two series of deflections—one ob-

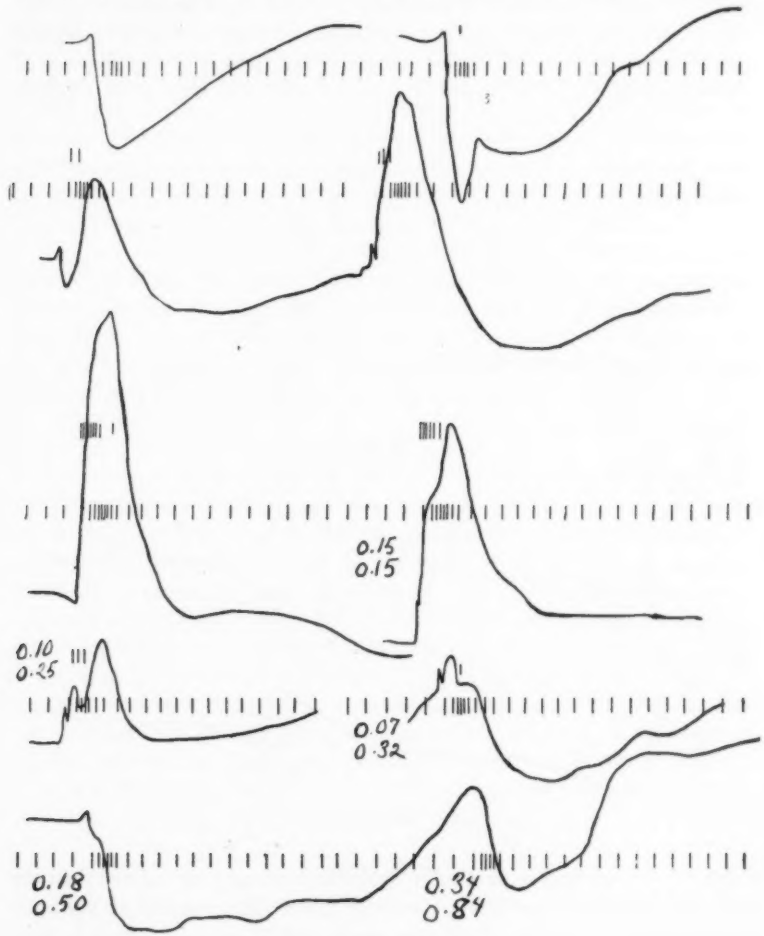

Fig. 8

tained with increasing strength of electrical stimulation and the other with decreasing strength of electrical stimulation—show dissimilarities, as demonstrated before, it is significant that the two series of deflections as obtained in this research have as many points in common as they do.

If the electrical method detects minute changes in metabolic activity we should be forced to conclude that a small injection of atropin may abolish visible secretion as normally elicited by stimulation of the chorda tympani, and yet permit activation of the gland as is indicated by the marked electrical deflection which may occur in the absence of visible secretion. This conclusion agrees with the later work of Barcroft (4) on oxidations in the submaxillary gland after atropinization and is in agreement with his theory of metabolite control of volume-flow of blood. Another conclusion we should be forced to draw is that larger injections of atropin may abolish all metabolic effects which the chorda tympani has upon the submaxillary glands. This conclusion agrees with the earlier results of Barcroft (3) on oxidations in the submaxillary gland after atropinization. If correct, it reduces the impor-

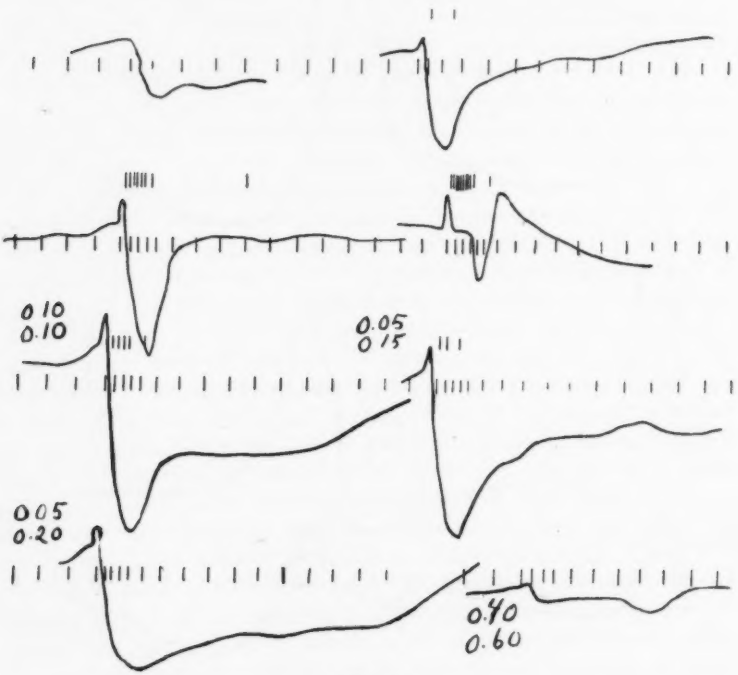

Fig. 9

tance of metabolite control, this control then only complementing the control by the vasomotor nerves.

In some experiments in which I have measured the oxidations of the gland as affected by stimulation of the chorda tympani after the injection of 3 to 5 mgm. of atropin (5), I found that although occasionally oxidations were increased as much as 15 per cent above the oxidations during rest, as a rule, oxidations were not increased by stimulation of the chorda tympani. (The Van Slyke method was used.) This amount of atropin usually abolished the electrical deflections. Oxidations were not studied after smaller injections. It would appear that the amount of atropin administered might markedly influence the results.

We know that living tissues, such as the secreting submaxillary gland, the thyroid gland, the kidney, and a nonglandular tissue such as muscle, exhibit electrical changes when their supply of blood is markedly interfered with. The inference might be that the electrical deflection is due to disturbed oxidation. In keeping with this inference is the observation that the metabolism of injured tissue is higher than that of normal tissue; on this basis the current of injury has been attributed to greater oxidations at the point of injury.

The delicacy of the electrical method of detecting metabolic activity has been demonstrated in many ways by Waller (6) in his researches on the *Signs of Life*. Yet it must be pointed out that a lack of change in electrical condition of a tissue need not necessarily indicate an absence of change of metabolic rate, for we know that a symmetrical structure such as the web of the frog's foot does not give an electrical deflection when excited symmetrically, whereas the single layer of skin of the back of the frog gives a large deflection. The magnitude of the deflection does not always vary in direct proportion to the rate of salivary secretion; in fact, occasionally the reverse happens. Apparently a balanced action of two effects of stimulation comes into play. There is, however, no evidence that symmetry of structure or perfectly balanced effects come into play more after atropinization than before.

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